CORNELL CONFERENCES
ON THERAPY

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CORNELL CONFERENCES ON THERAPY

VOLUME TWO

Edited by

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NEW YORK

THE MACMILLAN COMPANY

1949

Printed in the United States of America

Set up and electrotyped. Published, 1947.

Reprinted August, 1949

MOTTO

It is never too late to give up our prejudices. No way of thinking or doing, however ancient, can be trusted without proof.

HENRY DAVID THOREAU

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Introduction to the Series

The art of treatment represents the merger of two independent bodies of knowledge. In their development the two disicplines have followed separate lines, at times so far apart that little relationship between them is discernible. The science of pharmacology is often and properly concerned with totally impractical matters, and a large part of therapeutic knowledge is of necessity an anthology of purely empirical experience.

Forces appear to be at work which prevent their free interplay in the face of universal recognition of the fact that the best interests of medical practice are to be served only by their complete integration. It is the rare medical curriculum in which pharmacology and therapeutics are so arranged as to weave their teachings into a single and permanent design. The courses of pharmacology are isolated in a term or two in advance of the bulk of therapeutics and, to a considerable extent, clinicians continue to build the structure of therapeutic teaching with indifferent regard for the base already set for its support.

There is, in fact, in evidence a degree of competition and distrust between the two. Students are informed that thus-and-so is true in the treatment of the patient, in spite of what pharmacology offers to the contrary. There is the implication that the one is practical, the other theoretical and hence irrelevant. This attitude toward the relationship between pharmacology and therapeutics is more apparent in some schools than in others but none is wholly free of it. It is, of course,

unsound, for in any system of rational treatment the two are no more separable than the two faces of the same coin.

In ward and clinic the student is often told to do what in pharmacology he has been taught to avoid, and conversely, in pharmacology he often learns to expect what turns out to be alien to the experience of the clinic. It is quite clear that neither has taken full advantage of the opportunities afforded by the other. It is also clear that there is urgent need for a forum where pharmacologists and clinicians may come together and talk these things over .That, in essence, is the purpose of the Conferences on Therapy.

The Cornell Conferences on Therapy were inaugurated in 1937 as a joint venture of the Departments of Medicine and of Pharmacology. Arrangements were made for the participation of every department of the Cornell University Medical College-New York Hospital, and the collaboration of other institutions. They are scheduled weekly throughout the larger part of the year. It is the policy to begin on time and end promptly at the end of an hour.

There is considerable latitude in the conduct of the conferences. Certain features characterize the majority of them. A group of drugs, a therapeutic procedure, a symptom, or disease is selected as the topic for discussion. Practical procedures for the use of the therapeutic measure are outlined by a clinician, and a résumé of the experimental basis is presented by someone trained in physiology or pharmacology. Approximately half of the period is devoted to informal discussion in which the audience is encouraged to participate.

Free use has been made of the question as a particularly effective device for exciting interest and focusing attention. In some conferences the method of the "round table" discussion is employed, the questions being directed to a group of experts on the subject. The most successful conferences are among those in which the largest part of the session is devoted to informal discussion through the medium of questions and

answers. Those in which sharp differences of views develop and the evidence is probed, acquire a particularly stirring and stimulating quality. Therapeutic prejudice and vague opinion have a somewhat difficult time of it in these conferences.

The scope covers the whole range of therapeutics. To qualify for a conference, a subject must be a problem in therapy. It may be old or new. It should be important. If there are widely divergent views concerning it, so much the better, since it is the function of the conference to point out how the evidence stands. The order of subjects doesn't matter. A series of conferences in a particular field has been attempted from time to time, as one series on the treatment of the blood diseases. On the whole, it has seemed more practical to avoid the series on one subject, and to take up such topics as seem feasible in relation to their interest at the time and the personnel available to lead the discussion.

While the introductory remarks are often prepared, the discussion is for the most part unrehearsed and extemporaneous. In many cases the chairman tries to lead the discussion into a planned direction, but frequently the course is determined by the nature of the questions in which the audience appears to show the greatest interest.

The conference is no substitute for the formal lecture, the scientific article, or the textbook. It is not a substitute for any traditional form of medical teaching. It does not aim to treat any *subject exhaustively, but only to explore some aspects of* special interest, to analyze the evidence on controversial points of opinion and practice, to elaborate the physiologic and pharmacologic basis of therapeutic measures, and to present these on the level of the general practitioner.

There has been a good deal of experimentation in policy and technics. Certain features have survived: The purpose —to stimulate interest in rational therapy; the method—spontaneous, informal, and free discussion.

The conferences were originally designed for the students

of the third and fourth year classes of the medical college. It was soon discovered that members of the house staff, of the attending staff, and visiting practitioners had an interest in them. After the first year's experience, it seemed that a permanent record would enhance their value. It was anticipated that the reader might consider himself a participant. Accordingly, the conferences were taken down by a stenotypist in attendance at each session. The success of the edited record led to the next step, the introduction of the conference to a wider audience through their monthly publication in the *Journal of the American Medical Association* from 1937 to 1940, and since 1940 in the *New York State Journal of Medicine.*

Through their publication it was hoped that they might serve to demonstrate some of the advantages of this method of learning and lead to its adoption by other institutions. It is a method which can be readily adapted to the means and needs of small medical communities and hospital groups.

There has been widespread interest in the monthly publication of these conferences. From the large volume of correspondence and the nature of the comments, it has become clear that they are filling a need in medical education. Busy physicians find them a rich source of authoritative information in therapeutics, made more practical by the exchange of views among specialists and general practitioners, made more accessible by the restriction of their scope and the easy stirring style of the conference method.

In response to numerous requests, the final step in their evolution has now been taken, namely, the annual publication of a volume representing a group of conferences selected in the main for their quality and lasting value.

THE EDITORS.

Preface to Volume II

During the past year the Cornell Conferences on Therapy have been held regularly and in accordance with the policy and plan outlined in the "Introduction to the Series." They have continued to provide a forum for the informal exchange of views between specialists and general practitioners on problems of treatment. Their purpose is to promote the practice of therapeutics based on sound pharmacologic principles.

There are signs of an expanding interest in these conferences. Attendance has so enlarged that the former amphitheatre could no longer accommodate the audience. The sessions are now held in the largest lecture hall of the Medical College. *The American Journal of Medicine,* a new monthly publication whose policy it is to make more accessible to the general practitioner the specialized fields of clinical science, expressed an interest in printing the edited record of some of these conferences. Accordingly, the monthly publication system has been revised, one conference appearing every other month in the *New York State Journal of Medicine* and one every other month in the *American Journal of Medicine.* Volume I of the *Cornell Conferences on Therapy* has received a warm reception, and about eight months after its publication, it was found necessary to prepare for a second printing. All of these have encouraged the Editorial Board in the belief that the Cornell Conferences on Therapy are filling an important gap in medical education.

Volume II comprises a series of 16 conferences selected from the total of more than 100 which have been published

in the past few years. The choices were so made as to insure appropriate distribution of emphasis on new discoveries and basic principles, and with due consideration of the wide range of readers: general practitioners, specialists, surgeons, teachers, interns, medical students, pharmacists, nurses, and others.

In the past few years we have passed through a period of most intensive research in problems of therapy. Never in history have so many substantial advances been crowded into so short a space of time. Under such conditions it is extremely difficult for the busy practitioner to keep abreast; there is always the danger of losing perspective and mistaking shifting sands for solid ground. It is in precisely such a period that the Cornell Conferences on Therapy, through their free probings for evidence, proofs, and mechanisms, may render the greatest service in the practice of rational therapeutics.

THE EDITORS.

Acknowledgments

The Editors are indebted to the participants in these conferences, not only to those mentioned by name but to the large number of house officers, students, and visiting physicians whose names do not appear in the text. Their impromptu questions and discussions added greatly to the interest and liveliness of the conferences. The participation of members of the staffs of the Rockefeller Institute for Medical Research, College of Physicians and Surgeons of Columbia University, New York University College of Medicine, and New York Post-Graduate Medical School of Columbia University has been invaluable.

They are indebted to the many collaborators who assisted in the editing of the original stenotyped notes, especially to Dr. Janet Travell, and to Dr. Morris Pearlmutter for his aid in the preparation of the final manuscript of Volume II.

Grateful acknowledgment is also made to Dr. George W. Kosmak, Managing Editor of the *New York State Journal of Medicine*, and to Dr. Alexander B. Gutman, Editor of the *American Journal of Medicine*, for their kind permission to use material which had been published periodically in their respective journals.

List of Conferences

References to conferences: (1) N. Y. State J. Med. 46:1718 (Aug. 1) 1946; (2) N. Y. State J. Med. 45:744 (Apr. 1) 1945; (3) N. Y. State J. Med. 46:1246 (June 1) 1946; (4) N. Y. State J. Med. 46:2651 (Dec. 1) 1946, 46:2760 (Dec. 15) 1946; (5) Amer. J. Med. 2:86 (Jan.) 1947; (6) N. Y. State J. Med. 46:527 (Mar. 1) 1946; (7) N. Y. State J. Med. 45:519 (Mar. 1) 1945; (8) N. Y. State J. Med. 46:2303 (Oct. 15) 1946; (9) N. Y. State J. Med. 44.76 (Jan. 1) 1944; (10) N. Y. State J Med. 44:280 (Feb. 1) 1944; (11) N. Y. State J. Med. 45:65 (Jan. 1) 1945; (12) Amer. J. Med. 1:291 (Sept.) 1946; (13) Amer. J. Med. 1:539 (Nov.) 1946; (14) N. Y. State J. Med. 45:2309 (Nov. 1) 1945; (15) N. Y. State J. Med. 45:2515 (Dec. 1) 1945; (16) N. Y. State J. Med. 44:2606 (Dec. 1) 1944.

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CORNELL CONFERENCES ON THERAPY

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Jackson states that the physician may prescribe such preparations sometimes to patients who are malingering, and thus play one deception against the other until a correct diagnosis can be made. That is a statement that can be challenged. He implies that the placebo has no pharmacologic action, has no effect on the patient.

The best discussion, in fact, the only good one I found, is in Fantus' text, the 1939 edition. He states that the modern tendency is no doubt away from the placebos. It is not only more economical but more efficient to employ skilled, pure psychotherapy. He states that the lower the degree of the patient's intelligence, the more he may be benefited by a placebo. He goes on to state that millions are wasted by prescribing vitamins and fancy tonics. This brings out the point that there is a placebo element in most of the prescriptions of vitamins and fancy tonics. He adds that if one wishes to prescribe placebos, one should remember that iron and calcium are not very abundant in food. They might be deficient. They are cheap and inoffensive. He believes it is not right to cause anyone to pay for articles that have no value, and that it is best to write the prescription for something that has a recognized though slight remedial value. I am going to bring up that point later. Then he states that in the case of patients whose disordered imaginations torture them with afflictions from which they suffer quite as truly as they would from any real ill, it is clearly as much the physician's duty to employ such remedies as are likely to relieve the deluded imaginations,

Dr. Harold G. Wolff: This afternoon we are going to discuss a very important therapeutic device, the use of the placebo. Dr. DuBois will make the opening remarks.

Dr. Eugene F. DuBois: I think it is high time that we devoted a therapeutic conference to placebos. They have been considered the humblest, the most unscientific, and, perhaps, the most dishonest group of drugs that are used by doctors.

As a matter of fact, I think we can show that the study of the placebos is the most important step to be taken in scientific therapy. I am going to make several statements which will probably be challenged because they are drastic.

The first one is that, although placebos are scarcely mentioned in the literature, they are administered more than any other group of drugs. The second statement is that, although few doctors admit that they give placebos, there is a placebo ingredient in practically every prescription. The third statement is that the placebo is a potent agent and in its actions can resemble almost any drug.

As for the literature, we first ought to have a definition of placebo. It comes from the Latin word meaning "I will please," and its definition is "a medicine adapted rather to pacify than to benefit the patient." That is the definition given in the dictionary, adapted to pacify rather than benefit the patient. Well, does anyone believe that it is not benefiting the patient if we can pacify him?

I found extraordinarily little in the old literature about placebos. The *Index Medicus* for the last ten years does not

mention them. Practically none of the books on therapy mentioned it in their indices, but, I note that Clark in his 1940 edition of *Applied Pharmacology* states that drugs can be divided into two classes: placebos, which are given to tranquilize the patient, and substances which are intended to produce a definite pharmacologic action. He goes on to state that the use of placebos is psychotherapy and not pharmacology. I think that statement can be challenged.

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as it is to give certain medicines for a better defined disease.

Of course, the history of placebos goes back, way, way back beyond Hippocrates. They are the most ancient of drugs and we are safe in saying that in older times and in backward communities at the present time, about 90 per cent of the drugs which are given are placebos. They are inert drugs which please the patient and benefit the patient, and satisfy the doctor. The enormous success of homeopathy, where drugs are given in great dilution, in sugar pills, drugs so dilute that they could not possibly have any pharmacologic action, is a good example. Its success and therapeutic results are probably better than those in the case of some of the regular drugs that are given in huge doses by rival practitioners. At least, it has demonstrated very clearly what can be done by placebos.

I thought I would look at our *New York Hospital Pharmacopoeia* of the year 1816. That was the first pharmacopoeia issued, and it was not a bad one, by the way. There are lots of good drugs in it, and, apparently, these drugs were considered carefully by the doctors in this hospital over a hundred years ago. There is a list of about 160 drugs. If one examines these in the light of our present knowledge, I think about one-third of them would be considered inert. How about that, Dr. Cattell? Did you have a chance to go over that?

Dr. McKeen Cattell: Dr. Gold and I looked it over. That seems to be approximately correct.

Dr. DuBois: I had hoped to examine the *New York Hospital Formulary* of twenty years ago. I remember we gave it a pretty careful searching in committee, and I think that one-third to one-sixth of those drugs were inert. What is going to happen to our present hospital formulary when someone goes over it a hundred years from now? Well, I think most of the drugs have actions, but better drugs will be found by that time.

We can divide placebos into three classes, and I suggest this division. The first is the pure placebo, that is the bread pill or the lactose tablet. The lactose tablets have been found to be

as the red cell. *Frequently, too, platelets are surrounded by what looks like a definite clear outline, but a closer examination will show that the resemblance to a parasite is only superficial. The mass is granular throughout, a parasite is not. The staining is uniformly reddish or blotchy,* blue and red, it is not divided as in the parasite between a definite blue area and a definite red dot or dots.

Leucocytes, we have said, are also not uncommonly taken for the larger forms of parasites (*e.g.*, gametes), but only a beginner could possibly make such a mistake, as the leucocytes have a large densely staining mass of red (the nucleus) forming a considerable proportion of the whole cell mass, whereas in the gametes there is only a patch or so of red amidst the blue.

Dust on the eye piece is at once detected by rotating the eye piece when the body shifts its position.

The Normal Constituents of the Blood

Normal blood should be carefully studied in fresh and stained specimens.

1. *The Red Cells.*—With Romanowsky's stain these are only faintly stained reddish (Method 2), greenish or bluish in colour (Method 1). Their apparent size, *i.e.*, the area they occupy when flattened out, depends upon the thickness of the film: in well-made thin films they are large, and stain with beautiful uniformity. In fresh specimens (wet films) they ought to appear as perfect, uniformly straw-coloured discs; if crenated, it is impossible for the beginner to detect parasites.

2. *Leucocytes.*—The following types should be clearly made out in stained films:—

The Polymorphonuclear Leucocytes (Fig. 2).—These are very characteristic, and a reference to the diagram will make their recognition easy. It will be noted that they have a very irregular nucleus and fine granulations (stained red by ROMANOWSKY).

The Small Mononuclear Leucocytes (Fig 2).—These, the lymphocytes, are readily seen and can scarcely be mistaken for other forms. Their appearance varies somewhat with the thickness of the film. The thinner the film the larger they appear and the greater the area of protoplasm surrounding the nucleus. In typical forms the nucleus is dark staining and nearly spherical.

In wet films, a proportion of these cells shew a dark refractile spot (MANSON'S spot), which might be mistaken for a pigment granule, but, as we shall see later, they cannot possibly be confused with a typical pigmented leucocyte.

The large Mononuclear Leucocytes (Fig 2).—It is well to get thoroughly familiar with the appearance of this type of leucocyte, as the percentage of these is of great diagnostic importance in malaria.

Typical large mononuclear leucocytes are readily and clearly distinguishable. They are the leucocytes which may contain malarial pigment, and the recognition of a pigmented leucocyte gives a clear idea of their characters.

(i) They are in thin films of considerable size, half as much again to twice the size of the small.

(ii) The nucleus is large, oval, eccentric, not nearly so dense as in the case of the small—as is

shown by its less intense staining. They also present indentations giving a partly bi-lobed appearance.

(iii) The area of protoplasm surrounding the nucleus is considerable. It is clear, and contains at most a few scattered granules (ROMANOWSKY stain). The only difficulty will be found to arise in the case of a comparatively small number of 'intermediate' and 'transitional leucocytes.'

Intermediate Leucocytes (Fig. 2).—These are forms intermediate between the large and small mononuclear forms. They are usually classed along with the large forms, the characteristics of which they generally more nearly approach.

Transitional Leucocytes (Fig 2).—These are very characteristic, and when seen will be at once recognized. In shape, the nucleus approaches that of the polymorphonuclear forms, being trident shaped or S-shaped In consistence, however, it is obviously related to the nuclei of the large mononuclear cells. As a rule these cells are small in number, and from their close resemblance to the large mononuclears may be included with these.

Eosinophil Leucocytes (Fig 2). The large granules with which these are packed suffice to distinguish them. The granules are stained pink or blue (peripherally) by ROMANOWSKY. The nucleus in the eosinophil cells is frequently characteristic, consisting of two spherical portions united by a thin strand of nuclear material; it is really of the polymorphonuclear type.

Mast Leucocytes (Fig. 2, M).- These, in a film stained by ROMANOWSKY, are cells crowded with granules stained deep blue or nearly black.

These cells occur as isolated specimens in normal blood. They form about 0·5 per cent. of all white cells.

3. *Platelets* (Fig. 2).—Bodies of various sizes up to one-third diameter of the red cell, nearly always lying in clumps of from six to fifty, and stained bright crimson. They often show a considerable amount of differential staining, but differ entirely in appearance from parasites, more especially in having no *blue* stained mass. An isolated platelet lying upon a red cell may simulate a parasite. For the difference between it and a parasite, *vide* above, platelets often occur in large numbers in cases of malaria and, perhaps, especially in blackwater fever.

4. *Blood Dust or Granules.*—Small granules, smaller than micrococci. In fresh films they exhibit active motion (? Brownian).



Fig. 2A

Among abnormal constituents of blood we may mention:—

1. *Nucleated Red Cells.*—In conditions of loss or destruction of blood cells, *e.g.*, malaria, it is common to see nucleated forms of the red cell in the blood. They are characterized by a small globular nucleus with sometimes one or more little buds, staining almost black with Romanowsky. If the

film be counterstained with eosin, the fact that the surrounding pale area is red cell will become evident.

Two forms may be seen :—

(a) Normoblasts, *i.e.*, nucleated red cells the size of a red cell (Fig. 2A).

(b) Megaloblasts, *i.e.*, nucleated red cells *much larger than a red cell (Fig. 2A).*



Myelocytes

Fig 2B

Normoblasts are the form usually seen. Megaloblasts in excess are found in 'pernicious anaemia.'

2. *Deformed and Small Red Cells may be seen.*—These are known as poikilocytes and microcytes. They are common in severe anaemias, especially pernicious anaemia. It is quite exceptional to find deformed cells in blackwater fever. The red cells are generally quite normal in shape, though anaemic in varying degree.

3. *Abnormal Leucocytes.*—Under certain conditions, *e.g.*, malaria, but especially myelogenous leukaemia abnormal leucocyte forms are seen which normally are only found in the marrow, *i.e.*, myelocytes. These belong to the large mononuclear class, and may be of two kinds, either with large eosinophil granules as in the eosinophil cell, or fine neutrophil granules as in the polymorphonuclear leucocytes (Fig. 2B). If large

mononuclear forms are seen crowded with granules, films should be stained with EHRLICH's triacid stain, in order to accurately determine the forms of leucocyte present.

4. Frequently in malaria films (stained) large open meshworks of nuclear matter are seen *with little or no surrounding protoplasm.* These are degenerated or dropsical leucocytes, and often occur in great numbers.

5. *Red Cells with Long Wavy Processes.*—These are seen especially in anaemic bloods after the fresh film has been under examination for some time. They occasionally break off and float about. Shorter and more granular processes emitted by the red cell are even commoner.

6. Further, we must point out an extraordinary appearance of the red cells in stained films, so far as we are aware not hitherto described. *In anaemic (malarial) bloods, we find red cells, ten, thirty, or forty times the diameter of a normal cell, and these huge swollen structures shew at one side a crescentic area which is granular, and is the only remaining part of the red cell that can be recognized; the remainder is practically unstained. These gigantic structures may or may not be occupied by parasites.*

Chapter III

THE DETECTION OF THE MALARIA PARASITE

Examining The Film

After staining and drying, the film is ready for examination. *No Canada-balsam or coverglass need be applied.* A drop of cedar-wood oil is placed upon the film and the oil immersion lowered into it.

After the examination is completed, if it be desired to keep the film, the cedar oil is dissolved off by dropping a little xylol over the film and allowing this to drain off and then to dry. After drying, the film can be put away and kept indefinitely. If not needed the slide is placed on one side with others and eventually cleaned.

To Clean Dirty Slides

1. Rub with turpentine (benzine or xylol) to remove any adherent oil.
2. Wash with soap and water.
3. Rinse in water.
4. Dry and rub well with a clean cloth.

THE DETECTION OF THE MALARIA PARASITE

We propose to describe first the actual appearances which are likely to meet the eye, and later to give a systematic description and mode of distinguishing the various forms of parasite. A stained specimen (ROMANOWSKY) should always be used for the purpose of making a diagnosis.

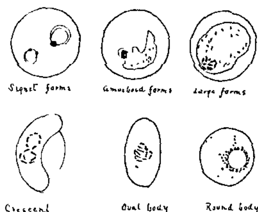


Fig. 3 Forms of the malaria parasite commonly met with in the blood —The dark dots in the first line represent chromatin, the fine dots, pigment.

We may first note that it is not necessary, as is often thought, to examine the blood at any particular time, but it is very necessary that the patient should not have taken quinine previously. Even five grains of quinine may so diminish the number of parasites as to make detection a laborious task, and a negative result under these conditions is not conclusive.

In examining the slide it is a very convenient method to begin at the edge of the film and to work systematically towards the 'tail' end.

The following forms of parasites may be seen:—

(i) Small forms looking more or less like rings, or stained streaks lying across or apparently stuck to the side of the red cell.

N.B.—Parasites free in the plasma are practically never seen.

(ii) Larger stained bodies of various shapes and sizes more or less filling the cell.

(iii) Crescents or large round or oval bodies with a cluster of coarse pigment placed more or less centrally.

1. *Ring Forms* (Fig. 3).—These may be quite small, one-sixth of a red cell in diameter, or much larger, one-third in diameter.

Rings are parasites of very distinct outline and structure. The part of the parasite that will first be noticed in a ROMANOWSKY specimen will be the red nucleus (chromatin), a clearly stained bright red dot (or dots). This is generally situated on the margin of the blue ring, which is equally distinct in outline, though often only a faint blue. The blue ring encloses an unstained vacuolic area. These rings stand out so sharply that they appear to project from the corpuscles. The red dot generally forms the signet of the ring (signet forms), but also may occur in the centre of the vacuole. The red nucleus or dot is often also rod-shaped or angular. The rings may shew a very faint blue outline or a thicker portion on the side opposite to the nucleus.

Though generally called 'rings,' these parasites are really discs, or saucer-shaped bodies, adhering to the sides of the red cells.

Besides these young rings, we have irregular forms of considerable variety, *e.g.*, a mere faint bluish line stretching across the corpuscle, yet always shewing somewhere a red nucleus, or a mere streak along the margin of the red cell, with, however, a red nucleus in the blue protoplasm (accolé forms).

Finally, no small structure should be diagnosed as a parasite unless it is clearly made out that it has three distinct parts.

(i) A red nucleus.

(ii) Blue protoplasm or body.

(iii) *A vacuolic area within the ring (in the* irregular forms this cannot be distinguished).

No confusion can then possibly arise with a platelet or stained vacuole or dirt.

A nucleated red cell has not these characters. Nor, again, has a red cell shewing polychrome or basophil staining, *i.e.*, a purplish or bluish mottling all over. In fact, no other body has the definite, quite easily distinguished characteristics of a parasite.

2. *Large Intra-corpuscular Forms* (Fig. 3).—They appear as more or less extensive areas of blue protoplasm, with one or more distinct, red areas. Pigment may be seen scattered over the parasite. These large forms are generally simple tertian or quartan parasites.

3. *Crescent and Crescent-derived Bodies*—These are most definite bodies, and readily recognized by the coarse pigment granules centrally situated. The presence of this pigment should absolutely

preclude the possibility of mistaking distorted red cells crescentic in shape, or a crescentic mass of platelets, for parasites. In neither of these is there a definite central pigment mass, nor should a foreign body be mistaken for a crescent. Moreover, crescents again have quite definite outlines, and shew a red-stained central portion and blue extremities.



Fig 3A Pigmented Large Mononuclear Leucocytes

The same criteria apply to the spherical form of the crescent.

4. *Pigmented Leucocytes* (Fig. 3A).—Large leucocytes with a large nucleus. Pigment (melanin) may occur scattered about the periphery of the cell, or in little clumps, or even in very fine powdery grains. The pigment is brownish-black in colour. Skin pigment may be seen in epithelium scales or free in the plasma, but the definite position of the pigment in the protoplasm of the leucocyte characterizes melanin

Appearances in a Fresh Specimen

1. *Rings.*—The very small forms of these are characteristic of malignant tertian infection. They measure about one-seventh the diameter of a red cell. A 'ring' is characterized by its rather opaque white look, its very definite contour, and

and in a moment three or four or more pale, long processes are emitted. The red cells all around are put in motion by their violence, and it may be only after a time, when the activity has grown less, that the flagella are actually seen. Nodosities will be observed in the flagella, and occasionally a speck of pigment at their extreme end. The flagella, after a time, break off, but they have only once, by MACCALLUM, been seen penetrating the female gamete.

Under certain unknown conditions the crescents do not become spherical and eventually flagellate, but remain as crescents.

Breathing on the slide, adding a trace of water, etc., have been recommended to produce the change more certainly, but it is probable that the real cause lies in the state of development of the crescent for certain observations, *e.g.*, those of Major BUCHANAN, I.M.S., shew that there is a certain time after the fever when a maximum number of gametes flagellate.

TO STAIN FLAGELLATED BODIES.

1. When flagellation is observed the coverglass is removed as carefully as possible, and slide and coverglass are then fixed and stained with ROMANOWSKY.

2. A number of rather thick drops of blood are placed on a series of slides. These are inverted over rectangular holes cut in blotting paper, moistened with water, and spread on a sheet of glass. A series of moist chambers is thus made. A dozen or more films are made, and each one is removed at intervals of five minutes, dried

(spreading out somewhat if necessary), fixed, and stained with ROMANOWSKY; or dry the thick film, decolourize with water, stain with ROMANOWSKY (without fixing), as in Professor Ross' method of making thick films.

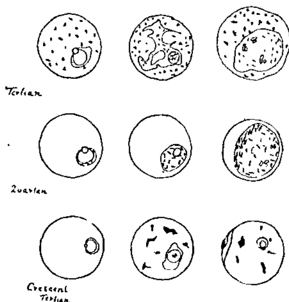


Fig 4 The three species of Malarial Parasites'

TO DETERMINE THE SPECIES OF PARASITE PRESENT

Three forms are recognized - simple tertian, malignant tertian, and quartan. The malignant tertian can, as we shall see, produce a quotidian

temperature with only a single generation of parasites. Whether or no there is a true quotidian parasite, one or more, is extremely doubtful.

1. Minute rings, *one-sixth to one-seventh* the diameter of a red cell, showing the signet ring shape, are characteristic of malignant tertian (Fig. 4).

2. Large rings.—If, when the temperature of the patient is still high, the rings are of considerable size, *one-fourth to one-third of the red* cell, they are, probably, simple tertian or quartan. If, on the contrary, the temperature is low and the febrile attack finished, these forms correspond to fully developed malignant tertian parasites.

The large *malignant tertian* rings have a characteristic appearance. They are *oval*, with a *thicker layer of protoplasm (blue) opposite the* nucleus (red).

3. Large forms, with considerable blue protoplasm and with pigment granules, are, probably, simple tertian or quartan.

The tertian parasite is an irregular and flimsy-looking body, and the medium sizes may show several pseudopodia (Fig. 4). Pigment is scattered throughout and is actively motile, while the quartan parasite is oval or globular, of compact appearance, with darker, coarser pigment, shewing but slow motion (Fig. 4).

The *enlargement of the cell* in which the simple *tertian* lies is also very *characteristic.*

In a well-stained specimen we have the further characteristic differences.

1. *Simple Tertian.*—The cell is dotted all over with fine red granules (SCHÜFFNER's dots), these cells strike the eye during the microscopic examination and are diagnostic (Fig. 4).

2. *Malignant Tertian.*—In specimens deeply stained with ROMANOWSKY, the malignant tertian parasite also produces changes in the red cell (Fig. 4). These consist of coarse dots or clefts, especially around the parasite. They are few in number and are equally characteristic of this parasite. Their appearance is quite different from SCHÜFFNER'S dots.

MAURER recommends the following method of developing them :—

10 drops of methylene blue (stock solution) + 25 c.c. of tap water.

15 drops of eosin (stock solution) + 25 c.c. of water.

Mix and stain for five minutes; shake actively the whole time.

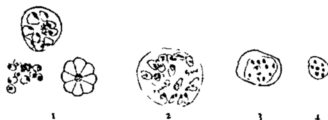


Fig. 5 1 *Quartan Parasites segmenting forms*
2 *Simple Tertian* "
3. *Malignant Tertian* "
4 *Quotidian (after ZIEMANN)* "

3. *Quartan.* The red cell shows no altered staining characters, but it may appear even smaller than normal. The parasite is not irregular in shape, but compact, oval, or globular. (In the fresh specimen the parasite is very refractile and has a peculiar opaque look) (Fig. 4).

The finding of crescents, is, of course, diagnostic of malignant tertian, but the possibility of a double infection *e.g.*, simple and malignant tertian must be borne in mind.

We have so far described the forms generally encountered during a febrile attack and the means of making a diagnosis, but it is necessary to consider other forms, *e.g.*, the sporulating forms, and more especially the gametes.

Sporulating Forms.—Besides the sporulating forms or segmenting forms, we can recognize also the presegmenting forms, in which the pigment begins to collect into a single mass and the chromatin gets split into a number of fragments. These are even commoner than the final or sporulating forms, in which the segments or spores are arranged around a central mass, though frequently here also the appearances do not correspond with the diagrammatic exactitude of the text-books. The segmenting and presegmenting forms are best seen in a case of regular quartan.

Quartan Sporulating Forms.—The pigment is placed centrally or often laterally, and grouped around it can be seen several, six to eight, chromatin masses. *In the presegmenting forms* the pigment has not yet condensed into a single blo. k, and the distribution of the chromatin masses is still irregular. In fresh preparations the typical 'daisy' forms can be clearly seen (Fig. 5).

Simple Tertian Sporulating Forms.—Here the whole parasite mass is larger, and fifteen or more chromatin segments can be distinguished (Fig. 5).

Malignant Tertian Sporulating Forms.—Rarely seen in the circulation. There are eight to ten chromatin masses (Fig. 5).

Gametes

Simple Tertian.—The young forms which, under certain unknown conditions, also appear in the circulation are characterized by the fact that the chromatin appears in the centre of the vacuolic area (Ruge), while in the asexual forms (schizonts) it is applied laterally.

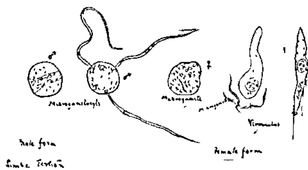


Fig. 6. Male and Female Gametes (after Schaudinn*)*

The full-grown gametes are much more easily distinguished. The female gamete (♀) is characterized by the possession of much protoplasmic matter, staining deep blue with Romanowsky and little chromatin; in the ♀ the chromatin is laterally placed, and is generally surrounded by a thin vacuolic area, the pigment is black in colour, and is irregularly scattered over the whole protoplasm (Fig. 6).

The male gamete (♂). The chromatin is more voluminous than in the female, it is of a looser texture, that of the ♀ being compact; the chromatin is centrally placed or extends in a broad

only very rarely indeed in the polymorphonuclear forms. As a rule, a pigmented large mononuclear (Fig. 3) is crowded with granules of pigment, the presence of only a few grains, or a single granular clump, is exceptional. The appearance of the clearly defined yellowish-brown or black pigment granules in the clear protoplasm is so characteristic, that no doubt ought to exist. It should be remembered, however, that in dirty films, specks of dirt may be over a leucocyte, and so resemble pigment. In this case, similar specks will be found lying free. The occurrence of malarial pigment free in the blood has never been seen by us.

Leucocytic Variation.—Often in cases where pigmented leucocytes are difficult to find, there is a very obvious increase in the percentage of the *large mononuclear leucocytes*. This change, which is usually very pronounced in the apyretic periods of an attack of malaria, is, however, most frequently absent during pyretic periods. If, during a period of low temperature, this change is not found, there is a strong presumption that the case is not malarial. If the blood be taken at the height of the fever, a negative result does not exclude malaria, and a further examination should be undertaken, if possible, during an apyretic period. In some cases, the change can be detected even during the pyretic periods, but in these it is always more marked in the apyretic. In some cases, during the course of the fever, no such change occurs, but appears immediately the temperature subsides, and diminishes as convalescence proceeds. Perhaps the cases where this test is of the greatest value, are those where the patient has

already been treated with quinine, and one can scarcely hope, even if the disease be malaria, to find parasites in the blood.

To Make a Differential Count of the Leucocytes

Large films are necessary, especially in malaria where, during the apyretic period, there is a distinct diminution in the *total* number of the white cells. It is important, in making films for leucocyte counting, that the margins and terminal points of the film be regular, and so in a convenient position for examination (Fig. 1). The margin of the film is focussed and passed beneath the objective. By passing along one-half or the whole of the margin of the film, the great majority of the leucocytes in the film are seen. In order to obtain accurate results, one thousand leucocytes should be counted, but a count of three or four hundred is generally sufficient for diagnostic purposes. Counts of a smaller number of leucocytes are valueless, as too great variations will occur.

As a leucocyte is seen, it is marked under the heading, large mononuclear, intermediate, transitional, small mononuclear, polynuclear, eosinophil, as the case may be. As many as ten to twenty or more are mentally noted before making each record in its column.

From the results obtained by blood counts of a considerable number of Europeans living in the tropics, we found that an increase beyond fifteen per cent. of the large mononuclear forms is proof of an actual or recent malarial infection, whereas

with a value of twenty per cent. it is almost always possible, by long search, to find an occasional parasite or pigmented leucocyte. A value of over twenty per cent. probably implies *actual infection at the time of observation.*

The Normal leucocyte values are :—

Polymorphonuclear leucocytes,		65-70 per cent.
Large *mononuclear*	"	4-10 " (Large mononuclear and Intermediate together)
Intermediate	"	
Small mononuclear *or lymphocytes*	"	20-25 "
Eosinophil	"	2-4 "

Other forms of leucocytes, *e.g.*, 'mast' cells, *are always in extremely small numbers in health* (0·5 per cent.)

Chapter V

THE PARASITE IN THE TISSUES

Tissues may be readily examined for the presence of parasites or pigment in the following way:—Place a minute portion of the tissue on a slide, and with the end of another slide spread it out as evenly and thinly as possible. Dry, fix, and stain in the same way as a blood film. Parasites, if present, are in this way much more easily and clearly seen than in sections. Spleen pulp, bone marrow, kidney, liver, etc., give beautiful results, and in the same way any secretion or fluid can be examined. For certain tissues, *e.g.*, bone marrow, it is advisable to fix in—

Absolute alcohol, 1 part,
Ether . . . 2 parts,

in order to dissolve out the fat present.

To Prepare Tissues

In preparing tissues for examination:—

1. Use as small pieces as possible, *i.e.*, at most five mm. thick.

2. Use plenty of the fixing and hardening fluid.

3. See that the separate pieces do not cohere to one another. Place some cotton wool on the bottom of the vessel.

Corked collecting tubes will be found most convenient and will hold ample material. The large masses of tissues sometimes sent home are of far less value to the pathologist than much smaller pieces well fixed and hardened. Always put a label *in the fluid*, with the data written on it in pencil, as well as the outside label.

Fixing

1. Alcohol is on the whole the most useful fixing fluid. Small pieces of tissue should be put directly into absolute alcohol. Larger pieces should be placed in ninety-five per cent. alcohol for two or three days, and then for twenty-four hours in absolute alcohol. Intestine should be spread on filter paper, as also nerves, or other tissue, which it is desired to keep flat. When removing the tissue from the paper, care should be taken that no fibres of the paper adhere, as they may prevent the proper cutting of sections.

For other modes of fixing *vide* appendix.

To Store Tissues

Keep tissues in diluted alcohol (seventy-five per cent. about). If kept long in absolute alcohol, many tissues become very hard.

To Embed Tissues for Section Cutting

Except for very special reasons, embedding in paraffin should always be the method employed. Very general misconceptions exist as to the *time*

and trouble necessary to prepare tissues in this way. It may be pointed out:—

1. That the times usually given for immersion in paraffin and other reagents are unnecessarily long.

2. That the use of two paraffins for embedding, a soft and a hard, is an unnecessary and even harmful procedure.

3. That an elaborate apparatus for the paraffin bath is unnecessary (*vide* later).

4. By using flat and very thin pieces of material, sections of considerable area may be obtained in a minimum of time. It is necessary to cut thin slices of the raw material (1 mm or less in thickness), and place these upon a small piece of paper before placing in the alcohol to harden. The paper keeps the slab from becoming distorted, and enables one to cut sections of the full area of the slab, say two-fifths inch square.

5. By placing minute pieces of tissue (in slabs on paper, if a section of some size is needed) directly into absolute alcohol, fixing, hardening, and dehydration can be accomplished within half-an-hour.

Necessary Apparatus for Paraffin Sections

1. *Cambridge Rocking Microtome.*—The ordinary form is all that is necessary, costing about five pounds. It is convenient to have a ball and socket adjustable holder, which enables one to change the angle of the block without remelting the paraffin.

2. *Razors.* These may be hollow-ground on one side, or on both, to a varying depth For

general use a *moderately hollow-ground* razor is used. Examine the edge under a low power to see if any notches exist, if so they must be ground out on a hone. A 'water of Ayr' stone, as long as possible, should be used and kept absolutely free from grit during use. The stone should be soft, capable of being scratched with a pin, and as a lubricant water or filtered kerosene oil may be used. After honing, the razor should be stropped. On one side of the strop a *minimum* amount of razor paste should be rubbed in and the leather side should be kept scrupulously clean and dry. If the razor is hollow-ground on one side only, it should only be honed on this side.

Examined under the microscope the edge should now present a clear, sharp line. It may be tested on a thin hair, which it should easily cut.

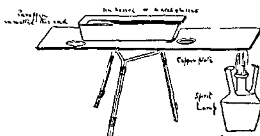


Fig. 8. Simple Embedding Apparatus

3. *Embedding Apparatus.*—A slab of metal (copper), 12 × 3 × ¼ inches. Heat this at one end, and place the vessel containing the paraffin at a point on the slab where the paraffin is just

kept melted. This is the temperature for embedding. This simple device serves all the purposes of an elaborate paraffin oven (Fig. 8).

4. *Alcohol.*—Absolute alcohol in the tropics has absorbed a good deal of water, and it is necessary to dehydrate it.

Heat crystals of $CuSO_4$ till a white mass is formed. Allow to cool, and place in a tall bottle of alcohol. Allow to settle and decant off alcohol as required. Add fresh anhydrous $CuSO_4$ if a marked blue tint develops, or tie up the anhydrous copper sulphate in a muslin bag, and place in the alcohol pot.

Gelatine may be used to dehydrate alcohol; it must previously be washed free from salts by soaking in water.

5. *Xyol.*—Xyol is the most generally satisfactory agent for displacing the alcohol and allowing the paraffin to permeate the tissue. Chloroform, wood naphtha, turpentine, oil of cloves, and other substances may be used.

6. *Paraffin.*—For use in the tropics, paraffin melting at sixty degrees will scarcely be found too hard. At high altitudes a softer will be required, and the right degree of softness must be determined and produced by mixtures of paraffin melting at 60° C. and paraffin of lower melting point, say 50° C., such as is suitable for use in temperate climates.

To obtain paraffin suitable for use in a given temperature place a block of paraffin in holder and cut thin sections.

(*a*) If the sections curl very much the paraffin is too hard.

(*b*) If the sections formed are forced together (telescoped) the paraffin is too soft.

(*c*) A certain amount of crinkling is usual with thin sections, and can subsequently be got rid of before mounting.

To Embed Tissues

1. *Alcohol.*—Several changes. If soft tissues, *e.g.*, liver, spleen, time is unimportant so long as dehydration is complete. If fibrous organs, the least possible time that will ensure dehydration (using thin slabs). Fibrous tissue becomes excessively hard if left too long in alcohol, xylol, or paraffin; thus skin and connective tissue require great care in preparation.

2. *Xylol.*—Ten to twenty minutes. When the tissues become transparent they are ready, and should be transferred without delay to melted paraffin.

3. *Paraffin.*—Ten to thirty minutes. If a tin trough be used, the tissues should not be allowed to rest upon the bottom of the trough, but be supported upon a strip of paper kept in place by folding the ends over the edge of the trough. A watch glass generally suffices.

4. Prepare a block for cutting by one of the following methods:—

(i) If the piece of tissue be small, smear a watch glass with glycerine, fill with melted paraffin and add the piece of tissue picked out of the bath with forceps, warmed by passing through the flame.

(ii) Fold a piece of paper, so that by folding a trough of required size is made. If an extra

length of paper be left at each end of the trough it can be folded down and holds the rest in position. Fill with freshly melted paraffin and add the piece of tissue picked up with warmed forceps.

(iii) Use metal pieces, supplied with most microtomes, upon a slab of glass.

The following points should be borne in mind:—

(i) Fresh paraffin should be melted for the block, as paraffin frequently melted, or kept melted for long periods, does not form so uniform a mass when cooled as freshly melted paraffin.

(ii) The more rapidly the paraffin is cooled, the more uniform is the resulting mass. It is well therefore, as soon as a well marked surface crust appears, to plunge the watch glass or trough into cold water.

When cold, cut out a square block with the tissue arranged in the position required for the sections.

5. *Cut sections.*

Note (i) The angle the knife is placed at is important, and must be found by experience.

(ii) It is well to use pads of paper to protect the edge of the razor, where it presses against the iron of the microtome.

(iii) To cut in ribbons, the top and bottom edges of the block must be parallel and horizontal. It is well to dip the block in soft paraffin, or merely to smear the top and bottom surfaces of the block with soft melted paraffin.

(iv) Cut as thin sections as will remain intact.

To Stain and Mount Tissues

1. If the sections are crumpled, float them upon water just hot enough not to melt the paraffin. They will become quite flat. Float the flattened sections on to a clean slide. Remove excess of water, and firmly press a piece of filter or blotting paper over the section. Thoroughly dry by holding a few minutes over the flame (care being taken not to melt the paraffin), or by placing for twenty-hours in a dessicator or warm oven. No further fixative is generally needed. If necessary, the slide may previously be smeared with egg-albumen fixative. It must, in this case, be dipped rapidly into the water and quickly withdrawn. (Appendix.)

2. If the sections are flat they may be placed *directly upon a slide slightly smeared with fixative.* In this case, celloidin in oil of cloves is the best *fixative. (Appendix.)*

3. Hold the slide or coverglass with the section over the flame till the paraffin melts. Dissolve off the paraffin with xylol, and then drop alcohol over the section. Place the slide or cover-glass in water.

4. *Stain.*—The best stains for general use are:—

(i) Haematein purissimus, saturated solution in 70 per cent. alcohol 10 cc.
Alum solution (alum 50 grammes, water 1,000 cc.) . . . 50 cc.

Stain for five to twenty minutes, according to the *depth of colour of the sections.*

(ii) Methylene blue, or gentian violet.

(iii) Counterstain, if desired, with watery eosin. For the detection of pigment it is well to stain a section faintly with eosin alone.

5. Pass through alcohol, oil of cloves, to Canada balsam. In hot moist climates, the cold produced by the evaporation of the alcohol causes dew to be deposited upon the slide. When the xylol or oil of cloves is added, this produces a troublesome milkiness and may spoil the section. To avoid this, all excess should be rapidly wiped up after the use of alcohol, and the oil of cloves added as quickly as possible.

Chapter VI

THE MALARIAL PARASITE

Life History

Among the groups into which the protozoa are divided we find such well-known classes as the Sarkodina, *e.g.*, Amoeba Coli, the Mastigophora, possessing flagella, *e.g.*, Trypanosomes, and the Sporozoa. It is these last that chiefly concern us. The Sporozoa include such orders as the Gregarines (*e.g.*, monocystis in the testes of the earth-worm) and the Haemosporidia (which include the malaria parasites of man, and blood parasites of birds, etc.) There is a close relationship between the coccidia and the haemosporidia (malaria parasite), the delevopmental cycles of the two being almost identical. The developmental cycle in the blood (the febrile cycle) of the malaria parasites was first demonstrated by Golgi, the further cycle in the mosquito by Ross. The cycle of Golgi is the asexual cycle, producing auto-infection of the patient ; the cycle of Ross is the sexual cycle, producing a new infection in a healthy subject.

The sexual cycle, it has been thought, commences in the blood when the conditions are unfavourable for the continuance of the asexual cycle, and, in fact, has been taken as a sign that the

the Vermiculus or Ookinet (Figs. 6 and 9). Both these terms are suitable ones, for the first describes the fact that the fertilized female becomes worm-like in shape, and the second that the fertilized

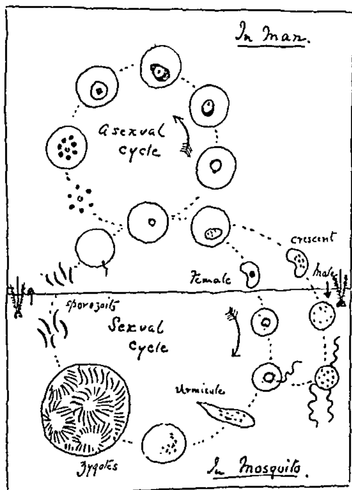


Fig. 9. Life Cycle of the Malaria Parasite in Man and the Mosquito.

;g moves. The vermiculus stage can be seen on ie slide in the case of halteridium, but in the case malaria parasites, only by taking the blood om the stomach of the mosquito after a suitable pse of time. The vermiculus now finds its way rough the epithelium of the stomach, and then es in the external muscular layers as a spherical ovoid body, the zygote. A kind of capsule is rmed around it by these tissues, and so at this age it is also called the *Oocyst*. Growth proceeds, id signs of division into several masses appear the protoplasm. This stage is termed a medium ygote or sporoblast. Then we reach the stage large zygote (or sporoblast), and by this time ie masses of the sporoblast have undergone ivision into a number of fine curved thread like odies, the sporozoits, so that eventually the large yst is almost entirely filled with sporozoits. he capsule of the cyst eventually ruptures, and he sporozoits pass from the tissues of the stomach o the thorax, being found at first amidst the nuscles, but eventually all collected in the alivary glands. From here they are injected into he blood by the mosquito, and they then attach hemselves to and penetrate the red cells (as has een actually observed under the microscope by SCHAUDINN), producing a new infection.

We may briefly summarize these various teps:—

1. Mikrogametocyte, and makrogamete in blood.

2. Development of mikrogametes = flagellation.

3. Fertilization of the makrogamete = ovum or copula.

4. Vermiculus or ookinet.
5. Zygote or oocyst.
6. Medium or large zygote = sporoblast.
7. Sporozoits.

The sexual cycle is known also as sporogony or amphigony, while the asexual cycle is known as schizogony or monogony. These two cycles and their relation to one another are shewn in the figure (Fig. 9).

Further, there is a certain amount of evidence to shew that a gamete in the blood can undergo a kind of retrogressive development, and give rise directly to young parasites (*i.e.*, schizonts). If this is so, it would explain the supposed function of old attributed to crescents (gametes) of producing relapses.

Chapter VII

MOSQUITOES

Mosquitoes belong to the order of Diptera, or true flies, which are characterized by:

1. A single pair of membranous wings.
2. Suctorial mouth.
3. Complete metamorphosis

In all mosquitoes, except the genera, Corethra and Mochlonyx, there is a long piercing proboscis, which is characteristic of the *Culicidae*. Mosquitoes usually are about five mm. in length, but certain species, *e.g.*, Megarrhina, are much larger.

Flies which may be mistaken for mosquitoes are:—

1. *Chironomus* – The chironomidae or midges are more delicate in structure than most mosquitoes, and are often bright green or pale yellow in

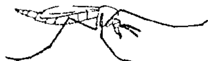


Fig 10. *Chironomus*

colour. They do not possess the characteristic proboscis of mosquitoes. The veins of their wings are more complex, and are *quite devoid of*

scales. The absence of scales upon the veins of the wings at once distinguishes these from true mosquitoes (Fig. 10).

Enormous numbers of Chironomidae are found near water, especially sedgy rivers and swamps. They are attracted by light, and are constantly seen around lamps and candles, a position in which true mosquitoes are scarcely ever found.

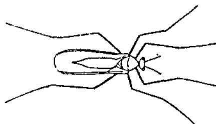


Fig 11. *Trichocera*.

2. *Trichocera*.—Some small Tipulidae often possess a considerable superficial resemblance to mosquitoes, as, for example, the winter gnat (trichocera). When at rest their bodies lie parallel with the surface, and upon it. They have no distinct proboscis (Fig. 11).

3. *Cecidomyidae*, or gall midges.—These have a simple wing venation, and there are no forked cells. In most species the wings and bodies are hairy, not scaled.

4. *Rhyphidae*.—These are readily distinguished on a close examination. Their wings are spotted.

Other flies than mosquitoes suck blood.

1. *Simulidae*, or sand-flies (sometimes also called midges).—These are minute flies which

suck blood voraciously. They have a short and stout proboscis. The salivary glands are very large in proportion to the size of the fly, and the bite is as severe as that of a mosquito. The males are harmless (Fig. 12).

The larvae of the Simulidae are aquatic, cylindrical in shape, and live in the stems of water plants. The imago hatches beneath the water.

2. *Phlebotomus* (family Psychodidae, or owl midges).—Small fluffy-looking flies which suck blood readily. They are most readily detected after feeding, when the abdomen is swollen with blood. They have very hairy wings and body, and a short powerful proboscis (Fig. 12).

3. *Tabanidae.* Large flies of heavy build (appendix, p. iv).



Fig. 12 Sand Fly (left) Owl Midge (right).

4. *Hippoboscidae.* Large flies of heavy build, inflicting a severe bite They do not lay eggs, the larval and pupal stage going on in the mother (appendix, p. vi)

5.—Tsetse flies (*Glossina*), etc. (*vide* p. 353).

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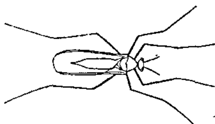


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5.—Tsetse flies (*Glossina*), etc. (*vide* p. 353).

LIFE HISTORY OF THE MOSQUITO

In common with all other insects shewing complete metamorphosis, the mosquito passes through four stages :—

The egg.
The larva.
The nymph.
The imago.

The Imago.—The imago is the well-known winged insect. The emergence of the imago may be seen on the surface of almost any collection of

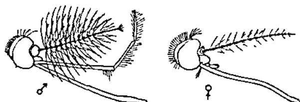


Fig. 13. *Heads of Male* (♂) *and Female* (♀) *Culex.*

foul water. Shortly after hatching, the insect may be seen resting quietly upon the surface of the water, and does not fly away when disturbed, or only very feebly. For some considerable time after hatching (twenty-four hours) the insects refuse to feed.

In the imago there are marked differences between the male and the female insect.

The Male.—In the male the antennae are markedly plumose. The palps also are long and hairy. The effect is to make the 'head' of the male mosquito very conspicuous (Fig. 13).

The male mosquito, with the exception of certain species, does not feed upon blood, and the proboscis is only used to suck in vegetable juices. The male of *Stegomyia* mosquitoes, however, sucks blood like the female.

The Female.—In the female the antennae are inconspicuous and have only short lateral hairs. The palps are also less conspicuous than in the male (Fig. 13).

The female feeds upon blood, and is frequently seen with the stomach distended with blood, more or less digested.

The female is also seen with the abdomen more or less swollen, with the greatly enlarged ovaries, which give a whitish and opaque colour to the mosquito, and often make the insect much more conspicuous in its flight than it otherwise would be.

The commonest species of mosquitoes belong largely to the following genera or closely related forms: —

1. *Anophelina* (sub-family).
2. *Culex.*
3. *Stegomyia.*
4. *Taeniorhyncus* and *Panoplites.*
5. *Uranotaenia.*

The sub-family, *Anophelina*, is in many ways the most distinct of these groups Not only are the adult insects highly characteristic in appearance, but the ovum and larva are quite unlike those of any other genus. One, indeed, can recognize the *Anophelina* at a glance merely by their characteristic general appearance, once the peculiarities of this genus are known.

upon a wall, its body projects so as to form a distinct angle with it. In some cases the angle assumed is almost a right-angle. In the case of almost all other mosquitoes, the body is held either parallel with the wall, or what is more frequent, the tail approaches the wall, giving the insect a 'hunchbacked' appearance. This difference is readily seen by any careful observer, and is a practical and useful distinction. A characteristic of *Anopheles* is that it rests by preference on the first two pairs of legs only, and keeps the last pair stretched out *stiff* and *straight*, or they slowly oscillate to and fro. Many mosquitoes wave the hind legs, notably *Stegomyia*, but they are held with the tarsi curved backwards.

The exact attitude adopted depends upon the species and the situation, whether a vertical or horizontal surface on which the *Anopheles* is resting. One very common species *(M. culicifacies)* at least, when sitting on a wall, looks exactly like a small brown *Culex*, since it holds its body parallel with the wall as a *Culex* does.

Culex.—Mosquitoes of the genus *Culex* are many of them brown mosquitoes of sober hue, *e.g.*, the common house *Culex*, *C. fatigans*, which is uniformly brown without markings. The genus, however, contains a very large number of species. In *Culex* mosquitoes the attitude when resting is 'hunchback.'

Stegomyia.—The genus *Stegomyia* is of the greatest interest and importance, since it is this form which is concerned in the transmission of yellow fever (*Stegomyia fasciata*).

These mosquitoes are generally black and white, with banded legs and abdomen, and spots

on the thorax. They are found in houses, and are most troublesome mosquitoes from their habit of feeding in the day, and their great alertness and persistence. *Stegomyia* are also very common in woods and forests.

Both male and female suck blood.

Taeniorhyncus (for exact distinctions, *vide* later p. 179).

Mosquitoes of the genus *Taeniorhyncus* are most frequently banded yellowish-brown.

They occur especially in the jungle, near swamps, river margins, etc., and in Africa, at least, may aptly be termed 'bush' mosquitoes.

Panoplites.—*Panoplites* are closely related to *Taeniorhyncus*. They are heavily scaled insects, almost floury in appearance. They occur especially near swamps, etc

Capture of Mosquitoes and Flies

1. Place a lamp upon a sheet of white paper, and note the insects which are attracted by the light Note insects belonging to the orders Lepidoptera (moths), Hemiptera (aphides, green flies, etc), Heteroptera (plant bugs), Neuroptera (caddis flies, stone flies, white ants). Pick out any mosquito-like flies. They will probably belong to the Chironomidae. Note the absence of proboscis, the delicate transparent structure. Note the plumed head of the male (as in the true mosquito) and the less conspicuous antennae of the female. Examine the wings under a strong lens or a low power of the microscope, and note that the wing veins are bare and do not carry any scales. Note that true mosquitoes are not seen around the lamp.

2. Examine, with a light, some wall which has been only dimly illuminated by the lamp, *i.e.*, some wall at the distance of several yards, and note true mosquitoes resting upon this. Capture several of these by placing a tumbler over them, and kill them by puffing in a little tobacco smoke. Observe that they have a distinct proboscis. Observe the 'plumed' male and the female without plumes. Examine the wings under a strong lens or low power objective, and note the scales attached to the wing-veins.

The specimens caught will probably be specimens of *Culex*. If near a swamp or jungly place there may be *Taeniorhyncus*, *Panoplites*, and possibly *Anopheles*. Observe the hunchback attitude in the case of most of the mosquitoes caught. If an *Anopheles* should by chance be caught, note the striking difference in the general appearance, the attitude, and the spots on the wings.

3. Observe in stuffy, furnished rooms, offices, etc., the presence of mosquitoes feeding actively during the day. Capture some of these. They will probably belong to the genus *Stegomyia*. Note their extreme alertness. Observe that they are black with white bands. Note the habit of waving the hind legs, and that the tarsi of these are kept curved. Ascertain whether the males feed upon blood.

4. Examine stables, huts, outhouses in the early morning.

LITERATURE

The Cambridge Natural History 'Diptera.' A most useful book for an introductory knowledge of a variety of winged life in the tropics and elsewhere.

Chapter VIII

THE OVUM

Ova are minute bodies *one mm. or less in* length. When first laid they are white in colour, but become rapidly brown or black. They occur on the surface of water, and if submerged do not



Fig. 16 Eggs of Stegomyia

hatch out. Mosquito eggs may be laid by the edge of water, or on floating objects, or upon the water. In the last case, they have some device to



Fig. 17 Egg Raft and Eggs of Culex

ensure that they shall float, and not sink and be destroyed. In the case of *Anopheles* and some species of *Stegomyia* (Fig. 16), each ovum lies

separately upon the water, and has air cells which keep it afloat. In the case of *Culex* and *Taeniorhyncus*, hundreds of eggs are cemented together to form rafts, each egg lying perpendicularly, with its larger end pointing downwards. In *Culex*, the

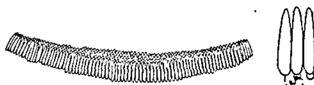


Fig. 18. Egg Raft and Eggs of Taeniorhynchus

egg-rafts are broad and roughly oval in shape (Fig. 17). In *Taeniorhyncus*, the egg-raft is extraordinarily elongated, resembling, in shape, a racing skiff (Fig. 18).

The Examination of Ova

Culex.—Examine the surface of some semi-putrid water for egg-rafts of *Culex*. Egg-rafts can almost always be found on the surface of water containing macerating leaves, fruit, etc. They are bodies of a blackish-brown colour, and are readily wafted about by the wind.

1. Note that the raft is boat-shaped, measuring one-fifth to one-third inch in length, and consists of two hundred to four hundred eggs.
2. Note that the separate ova are smooth elongated bodies, about seven to eight mm. in length. Note that there are no floats or other markings as in the case of *Anopheles* ova.
3. Note that one end of the egg is thicker and blunter than the other, and that to the thicker

end is attached a clear transparent globular body (the micropilar apparatus). Note that this body is readily detached, often leaving a spike-like process projecting from the thicker end of the ovum.

4. Note that the thicker end of the egg is placed downwards, and lies in the water. Ascertain by keeping the egg-rafts upon water until the larvae hatch, that the young larvae breaks through the lower end of the ovum.

5. Make as many observations as possible upon the egg-rafts, *e.g.*, time necessary for hatching of larvae, amount of desiccation they will withstand.

Anopheles.—The ova of *Anopheles* are difficult to detect in nature, but may be seen by the aid of a lens on the margins of small pools, where larvae abound. They are about 0·7 to 1·0 mm. long.

Examination of Anopheles Ova :—

1. Confine some female *Anopheles* as described on p. 120. Endeavour to choose those in which the ovaries are nearly mature (p. 97). Fifty to one hundred and fifty eggs are laid. Remove the piece of paper upon which the ova have been deposited and place this upon a slide Examine with a low power in strong daylight, and the mirror turned off.

2. Observe the remarkable resemblance of the ova to little boats, and the presence of the two beautiful oval air cells placed upon either side, acting as floats. (These are absent only in one species as yet described, viz., *M. turkhudi*). Observe also the presence of a white frill or a mere ribbed rim around what would be the gunwale of the boat (Fig. 54).

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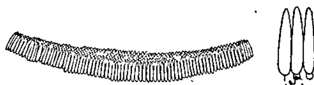


Fig. 18 Egg Raft and Eggs of Taeniorhynchus

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3. Observe that one end of the ovum is always stouter than the other. The stout end contains the head of the embryo, and is the head from which the young larva escapes. Note also that when *Anopheles* eggs are seen at the side of vessels drawn up by capillarity the thick end is at the bottom. Examine the surface of the water remaining in the hollow stopper or receptacle, and observe that the ova of *Anopheles* are laid singly without any cement substance, and float singly or touching one another on the water.

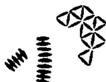


Fig. 19. Patterns formed by Eggs of Anopheles

4. Observe star-shaped patterns formed by some species, or the arrangement in parallel groups assumed by the ova of others (Fig. 19). Note that this arrangement is dependent on physical causes (shape of the egg, etc.), and not on the fact that the eggs are laid in such positions. This is readily done by stirring up a number of *Anopheles* ova on water, and noting how they tend to form groups in triangles and star shapes.

5. Ascertain that *Anopheles* ova, when first laid, are white, but rapidly darken and become black. Observe that *Anopheles* ova are very often laid in heaped-up masses, which eventually become dispersed by waves, etc. Observe that the eggs then form patterns.

6. Place some half-dried mud in a flat dish, and put this inside a piece of mosquito netting in which some *Anopheles* with ripe ovaries are placed. Observe that ova are laid upon the mud.

7. Preserve the mud for forty-eight hours, preventing it from becoming completely dry.

At the end of forty-eight hours or more, remove a few ova to a dry slide, and place under a low power. Allow a drop of water to flow on to the ova. Observe the escape, within a minute or so, of the young larvae, and the fact that a cap-like piece of the egg-shell is pushed off.

8. Observe that *Anopheles* kept in a dry test tube will occasionally lay their eggs on the side of the tube.

9. Note the time when the eggs were laid and the time at which the larvae emerge. This depends greatly on the temperature. It may take two to three days.

10. Remove *Anopheles* ova on paper and allow them to dry, and note that after two or three days at the most they will not hatch when carefully placed on water.

Stegomyia.—Confine some gravid females of *Stegomyia* mosquitoes.

1. Note that in some species the eggs are laid singly, and much resemble, at first sight, the ova of *Anopheles*. Note that in others the eggs are laid in rafts (*S. notoscripta*)

2. Note that they are irregularly oval, thicker at one end than the other, and have a corrugated surface in which are entangled numerous minute air bubbles.

3. Examine the surface of water left exposed for several days in a tumbler, etc. Note, if

Stegomyia mosquitoes have ovi-posited, the presence of eggs occurring singly or in parallel groups. Note that the ova are larger than those of *Anopheles*, and that they hatch into *Culex*-like larvae (see *Stegomyia* larvae, p. 85).

Taeniorhyncus.—Examine natural waters, especially small pools with a dense growth of alga, swamp pools, irrigated land, etc., for the egg-rafts of *Taeniorhyncus.*

1. Observe the extreme length and narrowness of the rafts. Note also how small a portion of the raft is submerged.

2. Observe that the ova are arranged as in *Culex* rafts with the thicker end downwards, and that they are smooth and have a micropilar apparatus.

3. Endeavour to obtain the ova of known species of *Taeniorhyncus*, by confining gravid females. Note the shape of the rafts.

Panoplites.— Observe that the eggs have a curious snout-like projection, and that they are laid singly.

Psorophora.—The eggs are large, two mm. long. They occur in patterns like those of *Anopheles*. The eggs are covered with minute prickly scales.

Observations upon the ova of the different species of mosquitoes are very meagre, and full and accurate descriptions of these are of great value (see Chap. XVIII).

Chapter IX

THE LARVA AND NYMPH

THE LARVA

The larvae of mosquitoes, more especially of *Culex*, are well-known objects. They can be seen by holding up to the light almost any specimen of water that has been left undisturbed for some days, but especially water which contains macerating leaves.

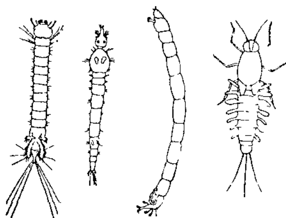


Fig. 20 *Larvae that may be mistaken for Mosquito Larvae*

Larvae which may be mistaken for those of mosquitoes are:

1. *Chironomus*.—The larva of Chironomus is a red worm-like creature (blood worms), and has no close resemblance to mosquito larvae (Fig. 20).

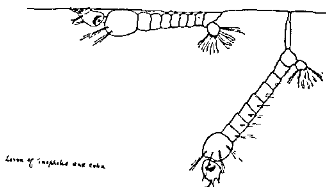


Fig. 21. Larvae of Anopheles (left) and Culex (right)

2. *Ephemeridae.*—The larvae of certain small Ephemeridae may, at first glance, be mistaken for mosquito larvae. There is no real resemblance, and the triradiate tail of the ephemera larva at once distinguishes it (Fig. 20).

3. The larva of dixa rather closely resembles the larva of *Anopheles*, though not other mosquito larvae (Fig. 20).

Examination of the Larva

Culex (Fig. 21).—Obtain some Culex larvae from any source and place in a glass vessel.

1. Observe the hanging attitude of the larva. Note the angle it makes with the surface of the water, and how this varies in different species.

Note, if the larva of C. *concolor* is being examined, that the position is nearly horizontal.

2. Observe the large head, the prominent eyes and projecting antennae.

3. Note the long respiratory syphon arising from the eighth abdominal segment.

Place a half-grown larva under a coverglass and examine under one-third inch objective.

1. Make an accurate drawing of the antennae.

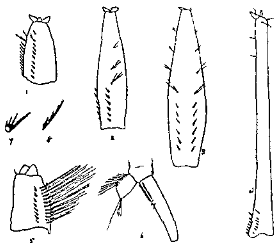


Fig. 22 Respiratory Syphons of Larvae
(1) *Stegomyia*, (2) *Culex* (3) *Culex with Large Syphon Tube*, (4) *Taeniorhyncus*, (5) *Cannibal Larva* (? C *Concolor*), (6) *Enormous Syphon Tube (one-quarter scale of others, genus undetermined)* (7) *Spine on Tube seen on the flat* (8) *Spine seen sideways*

2. Carefully observe the length and thickness of the respiratory syphon.

3. Note the absence of palmate hairs.

4. Determine whether the larvae examined are *Culex*, *Stegomyia*, *Taeniorhyncus*, etc.

straight alimentary canal. Place in a drop of clean water and crush under a coverglass. Note what organisms form the chief bulk of the food. Note the presence of sand grains—unicellular plants and animals—short lengths of alga, diatoms, etc. Also bacilli.

Determine the common foods of several species of *Anopheles*.

4. *Culex Larvae.*—The larvae of the *Culicidae*, with the exception of those of *Anopheles* and possibly some other genera, are superficially much alike. The conspicuous hairs and spines, and even the complicated terminal segment, are very similar in the different genera. There are, however, marked differences in some features on closer examination. These differences are mainly to be found in the syphon tube, the antennae, and mental plate, but to a less extent in other structures.

Examine some Culex Larvae.—Note differences in naked eye appearance; note the long worm-like *Stegomyia* larva and its wriggling mode of progression; note the transparent and spiny appearance of some larvae (notably *Taeniorhyncus*); note that some larvae adopt a nearly horizontal attitude (*C. concolor* and others), others a vertical attitude (*Stegomyia*), whilst the majority form a small angle with the vertical. Examine larvae under a low objective. Note the head with eyes, large feeding brushes, antennae and various hairs; note the large bunches of hairs arising from the thorax and abdominal segments; note the last segment bearing four large clear papillae and two systems of hairs; note the penultimate segment which carries the syphon tube and some curious claw-like spines.

Note especially the following :—

(i) The syphon tube.
(ii) The antennae.
(iii) The mouth parts.
(iv) The anal papillae.
(v) The hairs of the thorax and abdomen.

The Syphon Tube.—This is formed of a single cylindrical piece of chitin, and contains the origin of the two main tracheae of the body. Note the small flap-like pieces of chitin forming a closing apparatus at the extreme tip. Measure (by eye-piece micrometer) the length and greatest breadth of the syphon tube, note that in different species, and especially in different genera, the syphon tube varies greatly in its measurement By dividing the length by the breadth a figure may be obtained which is useful, and may be termed the syphonic index number, note that in *Stegomyia* this number is about two In *Culex*, four to seven. In *Taeniorhyncus*, as much as twelve in some cases. Draw accurately by measurement (eye-piece micrometer) a number of syphon tubes of different *Culex* larvae. Note that marked variations in different species exist.

Note two rows of spines on the posterior aspect of the syphon tube, starting from the base and extending a variable (in different species) distance up the syphon tube, note that they differ in number and length, etc., in different species. The spines appear serrated or compound, according to the angle they are viewed from, and differences may be supposed to exist which depend upon this fact. In some species (certain carnivorous or cannibal larvae) a large fan of hairs projects posteriorly in the medium line from the syphon

tube. In certain species the syphon tube is of enormous size, and may attain to one-third the length of the larva.

The Antennae.—Note in the case of most typical *Culex* larvae that the antennae are large conspicuous objects: note a basal, medial, and terminal portion, and a large bunch of feathered hairs arising at the junction of the two first-named portions; note also large single and stout hairs from the more terminal portion; note spines on the body of antenna.

Examine the Antennae of various Larvae.—Note in some cases that the antennae are more rudimentary (*Stegomyia*, *Anopheles*). In the case of *Stegomyia* (as far as described) they are small and spineless, and possess only a small hair arising from a papilla, which may be single or in three or four branches. Make drawings (using eye-piece micrometer), and note great variation in different genera and species.

The Mouth Parts.—Note the characters of the claw-like mandibles, and especially the exact character of the triangular mental plate, which forms a conspicuous dark triangular body on the under surface of the head.

Note that in different species the plate varies in appearance, especially in the size and number of notches in its margin. In some species the plate is like a shark's tooth, in others it is comb-like.

The Anal Papillae.—Note the tracheae ramifying in these, the papillae being possibly gill-like in function. In most species they are pointed, in others they are globose at the end.

The Large Body Hairs.—These are long in some larvae, much shorter in others; their arrangement is very similar in the different larvae.

5. *Cannabalism of Larvae.*—Add some large *Culex* larvae to a small bottle containing some small larvae or *Anopheles* larvae. The *Anopheles* larvae or small *Culex* larvae will be devoured by the large forms.

6. Observe the occurrence in nature of the two forms, *Culex* and *Anopheles*, also what *Culex* larvae are found living together.

7. *The Enemies of Larvae.*—Add small fish, waterbeetles, and their larvae (Dytiscidae, Hydrophilidae), libellula larvae, corysca, nepa, tadpoles, and other water animals, respectively, to a series of wide-mouthed bottles containing equal numbers of larvae. Note the rate at which they are devoured, if at all. The carnivorous forms nepa, corysca, libellula rapidly devour larvae. Hydrophilidae beetles, tadpoles, etc., do not destroy larvae. Observe that some species of fish are much more active devourers of larvae than others. Note that weeds often protect larvae from being consumed by small fish.

8. Make experiments with different chemical and other bodies, and note the absence or presence of culicidal power.

(a) Note that chemical bodies in solution kill only with difficulty, as a rule, *e.g.*, corrosive sublimate. Ammonia, however (1 in 4,000 of water) will kill mature larvae according to WADDELL.

(b) Note that oils rapidly kill larvae by blocking the air tubes. Treat larvae by pouring a little olive oil upon the water Stain with osmic acid and note globules of oil within the air tubes.

9. Add some paraffin oil to a small *Anopheles* pool, observe the presence next morning of dead female mosquitoes that have come to lay their eggs. Observe the effect of paraffin on different kinds of natural water, and the great efficacy in some cases and futility in others.

10. Observe that pools covered with Lemna are very frequently, if not always, free from larvae. The action of the Lemna is said to be mechanical.

Examination of other Larvae

Dixa.—In its movement along the surface of the water the larva of *Dixa* resembles *Anopheles* larvae, and this larva also rests horizontally just beneath the surface film.

In *Dixa* there is no globular thorax, and the whole larva is longer and thinner than an *Anopheles* larva (8 mm.) Moreover, *Dixa* larva only indents the surface film at the head and tail, there being no palmate hairs on any of the segments. *Dixa* larvae move very rapidly, and have a habit of climbing above the surface of the water and resting in a loop with the head and tail downwards. When placed in a specimen tube it climbs up the side and becomes lodged in crevices in the cork.

It is found frequently in running water (*Fig. 20*).

Mochlonyx Larva.—Note absence of palmate hairs on dorsum and presence of respiratory syphon, absent in *Anopheles*. They are extremely voracious. They lie deep in the water.

Corethra Larva.—These are the so-called 'phantom' larvae. They are extremely transparent, and lie horizontally rather deep in the water. The head is smaller than in *Anopheles.* There is no air system communicating with the external air. They are extremely voracious. Add some *Corethra* larvae to a tumbler containing *Culex* larvae.

Stegomyia.—The larva of *Stegomyia* is rather longer than that of *Culex.* When disturbed it exhibits a rather lashing movement like that of certain small aquatic worms. When at rest at the surface, the attitude of the body is almost vertical.

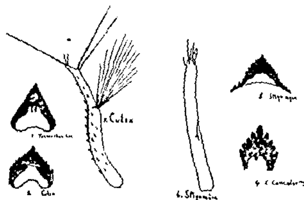


Fig 23 Antennae and Mental Plates of Larvae

The larva, however, spends a good deal of its time browsing at the bottom of the water, and then lies for the most part horizontally.

The head is small in proportion to the rest of the body, and the thorax is less conspicuously marked off from the abdomen than in *Culex*

The antennae resemble those of *Anopheles* larvae, more than those of *Culex*. The large branched hair of *Culex* is represented by a short inconspicuous simple hair (or as many as three) projecting from the side of the antennae (Fig. 23).

The syphon tube is characteristic, being very short and stout (Fig. 22), only twice as long as broad, whereas in *Culex* the syphon tube is four or more times as long as broad (Fig. 22).

1. Examine domestic utensils, disused water pots, and tins containing water. Examine the water which collects in the axils of the banana leaves, collections of water in tree-stumps in the jungle. If larvae are present—

(i) Note the very short and broad spiracle tube and its dark colour. Compare with that of *Culex* and *Taeniorhyncus*.

(ii) The larva is longer and more worm-like than most mosquito larvae. The 'wriggling' motion is also very markedly shewn owing to the length of the body.

Taeniorhyncus.—In natural waters, especially shallow trickling water forming pools, with a dense growth of spirogyra, etc., swamp water and river margins, the larvae of *Taeniorhyncus* will be readily found.

1. Note that the larva lies often embedded in the masses of green spirogyra or other thread-like algae.

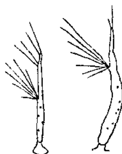
2. Note the great transparency of the larva and the frequency with which brilliantly green specimens are found.

Under a low objective note the following, which appear to be characteristic of this genus:—

1. The enormous horn-like and curved antennae (Fig. 23A).

2. The extreme length and slenderness of the syphon tube (Fig. 22).

Psorophora.—The larvae are large, half-an-inch in length. They are extremely cannabalistic.



Culex Fatigans *Taeniorhyncus*
Fig 23A

Information upon the larvae of the different genera of mosquitoes is so meagre, that in as many cases as possible the larvae of different species should be determined, and systematic descriptions and drawings made of such important parts as the antennae, syphon tube, and such other parts as may be found to vary in the different larvae observed

NOTE—Further work on the syphon tubes of larvae has shewn us that they are of *specific* as well as generic importance. The extremely long and slender syphon tube is apparently not confined to *Taeniorhyncus*, but mosquitoes having this type of tube have always a *banded proboscis*.

The Nymph

The nymphae of mosquitoes are extremely characteristic bodies. Wherever a number of fully-developed larvae are found there will generally also be seen numbers of bulbous comma-shaped creatures, having a large globular body (head and thorax) and a small tail, kept more or less tucked in beneath. When disturbed they dart downwards with great speed, but very soon reappear at the surface.

Nymphs are not so easily seen in pools as larvae.

The differences in the nymphae of different genera of the *Culicidae* is not nearly so great as in the case of the larvae.

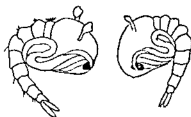


Fig 24. *Nymphae of Anopheles and Culex*

By keeping under observation a number of nymphae, some will be seen to become less inclined for active movement, and the abdominal segments (tail) may be extended horizontally. Soon after these changes the adult insect emerges through a crack in the chitin of the back of the

thorax. The process as seen in *Anopheles* is very fully described by NUTTALL and SHIPLEY.[1]

EXAMINATION OF NYMPHAE

1. Note the effect of tapping the glass vessel and the rapidity with which the nymphs regain the surface.

2. Observe that when first they appear the nymphs are light in colour, but darken very considerably later.

3. Note that just before the hatching of mosquitoes the nymph lies with the tail extended, and that silvery marks may be seen, due to air lying under the chitin.

4. Observe the emergence of the imago.

Examine the nymphs of *Anopheles*, *Culex*, *Taeniorhyncus*, etc., and observe that to the naked eye they are very similar.

1. Note that the nymphae of *Anopheles* lie less vertically in the water than those of *Culex*. ✓

2. Observe that the nymphs of *Anopheles* are more elongated antero posteriorly and compressed laterally than those of *Culex* and *Taeniorhyncus*. ✓

3. Observe the very large nymphs of some common species of *Taeniorhyncus* and the great length of air tubes which are directed straight forwards in a very characteristic manner.

Place nymphae in drops of water on a slide and examine the air syphons. Note—

1. In *Anopheles* the syphons have a square truncated end, and are proportionally much shorter than in *Culex*, and project from about the middle of the thorax (Figs. 24 and 25). ✓

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1 *Journal Hygiene*, vol 1, part II

2. In *Culex* the syphons are long and narrow, and have a slit-like opening, and project from the posterior portion of the thorax (Figs. 24 and 25).

3. In *Stegomyia* the syphons are broadly triangular, and are characteristic. Note the marked contrast in appearance to those of *Culex* (Fig. 25A)

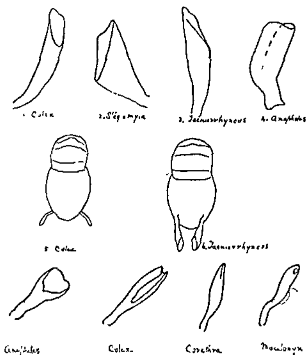


Fig. 25. Nymphal Syphon Tubes

Examine the nymphs of *Corethra* and *Mochlonyx* when they are encountered.

(*a*) Note in *Corethra* the pointed syphons and the straight tail (Figs. 25 and 26).

(*b*) Note in *Mochlonyx* the *Culex*-like nymph and the thin rounded and pointed syphons (Fig. 25).

Examine the bottoms of pools of polluted water, and note in the mud the brilliant red nymphs and larvae of *Chironomus* (Fig. 26).

1. Observe that the *Chironomus* nymph has a large globular body (head and thorax) and bears a general resemblance to mosquito nymphs.



Fig. 25A. Nymphal Syphon Tube of Stegomyia

2. Note, however, the presence of the conspicuous white feathery gills which form tufts at the side of the head.

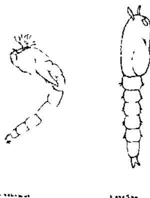


Fig. 26. Nymphs of Chironomus and Corethra

3. Note that the *Chironomus* nymph and larvae do not rise to the surface to breathe as do those of mosquitoes.

4. Note the curious rhythmic bending movement of the larvae and nymph of *Chironomus* which, when they are present in numbers, give the mud at the bottom of the pool a curious appearance.

LITERATURE

Miall. *Aquatic Insects.*

Chapter X

TO CAPTURE, PRESERVE ALIVE, AND EXPERIMENTALLY FEED MOSQUITOES

To Capture Anopheles

Necessary Apparatus.—One or two small collecting tubes (Fig. 27), a clean and perfectly dry bottle (whiskey bottle), some cotton wool.

To Detect Anopheles

Choose a suitable native village, *i.e.*, in Africa, any bush village; or in India, any village near a nallah or other source of *Anopheles* larvae

Determine whether *Anopheles* exist in any of the following situations:—

1. In the dark corners of sheds, cow-houses, or other out-houses.

2. Under the eaves (in darkish parts) of the huts.

3. In the huts themselves, hanging to straws, stalactites of soot, etc., etc.

4. Any other likely situations, *e.g.*, collections of dry grass; in the undergrowth in the bush (capture in this situation is difficult).

Procedure.—If, on inspection, none of the insects can be detected by careful scrutiny (the

most concentrated attention is, as a rule, needed), the thatch should be carefully disturbed with the hand or a short stick.

Observe carefully any insects which fly out, and note where they settle. Choose especially portions of the thatch which are not too dark to prevent one seeing clearly, but are not too much exposed to light.

Train, if possible, one or more intelligent natives to detect the insects and to collect them, as shortly described. It is a good thing, if even only a very few *Anopheles* have been found by a personal inspection, to offer a small reward to any persons in the village who will undertake to collect them. One or two tubes should be left for this purpose.

To Detect Culex

Examine the walls of houses, out-houses, and native huts. Especially examine clothes hung up in native huts. Many specimens of *Culex*, resting in their characteristic hunchback attitude, will probably be detected. Especially on dark clothing, old blankets, inside leather boots or boxes.

Mosquitoes seem especially fond of the smell (?) of leather.

To Detect Taeniorhyncus

These are best caught by sitting, with a light, near a marsh or grassy land. A wall, or tent, or cloth hung up should be at hand, and kept slightly illuminated with a lamp. They may be captured as they settle upon the sheet or upon oneself.

To Capture Mosquitoes

1. Place a collecting tube *very slowly* over the mosquito.

2. Insert a finger underneath, and so rapidly block the tube; or a piece of cardboard or wool may be carefully slipped underneath.

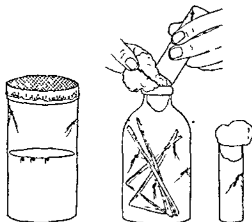


Fig. 27. Method of Collecting and Breeding-out Mosquitoes

3. Place a plug of cotton wool in the mouth of the tube.

4. Transfer to the large bottle by placing the tube over the mouth of the bottle and withdrawing carefully the cotton wool. Keep the bottle closed with a plug of cotton wool.

5. Capture as many specimens as required, and transfer them as caught to the bottle.

A wide-mouth bottle, over which a piece of stout paper has been tied, with a small trap-door cut slightly larger than the tube, may be used. This has the advantage that mosquitoes do not so easily fly out into the tube during the act of transference. It has the disadvantage that the paper tears, and the mosquitoes are more likely to escape through accidental circumstances.

To Breed Out Mosquitoes

(Fig 27)

Collect a number of full-grown larvae and nymphae of both *Anopheles* and *Culex*.

1. Separate the nymphae from the larvae and place them in a jar or wide-mouthed bottle half-full of water, leaving room for the insects when hatched. Cover the jar with a piece of thick cardboard or a lid, the central portion of which is replaced by mosquito netting.
2. Place the larvae where they will receive plenty of light, but will not be subject to great heat.
3. Remove the nymphae as they are seen at the end of each day.

To Keep Mosquitoes Alive

The length of time mosquitoes remain alive in captivity depends almost entirely upon the suitability of the conditions under which they are kept.

Except for special purposes, mosquitoes (especially *Anopheles*) should *not* be kept in open spaces, *i.e.*, frames covered with mosquito netting.

Procure several 'chutney jars' with hollow

glass stoppers. These can be obtained generally from the native bazaar in India for a few annas (Fig. 28). This form of jar is very convenient, but any other jar will serve.

Cut a piece of thick cardboard so that it will, when forced down into the jar, remain supported on the shoulders of the jar.

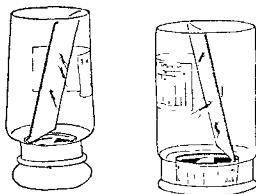


Fig. 28. Method of keeping Mosquitoes alive

Fill the stopper nearly to the brim with water. Cut a thin slice of cork and place it on the surface of the water. Upon the cork place a piece of clean white paper. The paper should not quite occupy the whole of the space in the mouth of the stopper.

1. Invert the chutney jar (prepared as above) over the top of a jar in which some mosquitoes have hatched. Remove the cardboard and gently tap the glass. The mosquitoes will fly upwards into the chutney jar. Place the chutney jar containing mosquitoes upon its stopper prepared as above.

Place the whole, after labelling, *in a dark cupboard* or other convenient place (incubator).

At the end of the first day or so, the males will be found dead upon the piece of paper, and can be removed. On the second night after hatching, most of the insects will feed, and the jar is ready for use.

2. Place the inverted (chutney) jar, prepared with cardboard as above, over a bottle in which *Anopheles*, caught in a village or elsewhere, *have* been placed. Remove the cotton plug and shake the bottle gently to drive the insects out. Replace jar upon the prepared stopper. Place in a dark spot. Next morning remove the stopper and remove *any dead mosquitoes and ova by taking out* the piece of paper.

On the second night after the mosquitoes have been collected, the bottle is ready for feeding experiments. On the third day, generally, the mosquitoes have no longer any blood remaining in the mid gut, and are ready for dissection.

The glands of any mosquitoes that may die before this may of course be dissected, if desired, on the chance of finding sporozoits.

In the use of village-caught *Anopheles*, it must be borne in mind that any subject upon which they are fed is liable to a fresh infection. In the case of natives (who sleep without hesitation in any village), the employment of village-caught mosquitoes cannot, however, be very prejudicial.

The advantages of the above way of keeping mosquitoes are :—

1. The mosquitoes will keep alive longer than in any other way known to us.

2. The immense convenience in feeding.

3. Any mosquitoes that may have died in the night can be recovered, and are not dried up.

4. It is an extremely convenient way of obtaining and examining the ova.

5. Mosquitoes which have become feeble are given the best possible chance of living, and will be found resting all day on the piece of paper.

If boxes and net covered frames be used, an enormous mortality usually results. The dead *bodies dry up and get lost in the folds of netting*, or, unless special precautions are taken, are eaten up by ants.

If chutney jars with hollow stoppers cannot be procured:—

Procure any form of wide-mouthed jar or bottle, such as a prune jar, preserved fruit bottle, etc. Insert a piece of stout cardboard as before.

1. Prepare the metal top of a screw-top bottle or some other suitable small receptacle with water, cork, and paper as above. Place upon a square piece of very stout cardboard or wood. Invert the jar over this (Fig. 28).

2. Prepare a saucer by adding a few teaspoonfuls of water and placing on this cork and paper. Invert the jar over the saucer. This is rather more convenient than the last mentioned method, as mosquitoes are less liable to escape in the process of lifting the bottle.

To Feed Mosquitoes

Select a bottle in which the mosquitoes (twenty to thirty, or at least a dozen, in each bottle) are ready for feeding, *i.e.*, the second evening after hatching or collecting. Lift the bottle from the

stopper, first disturbing any mosquitoes which may be resting on the stopper, and place it mouth downwards on the table.

Slip underneath the mouth of the bottle a small piece of mosquito netting of rather a fine mesh. Tie this around the neck of the bottle with twine. The bottle is then ready for feeding.

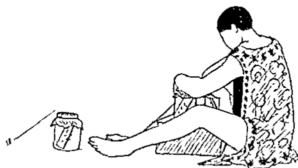


Fig. 28A. Method of Feeding Mosquitoes

At, or shortly after dark, take as many bottles as may be desired to the ward or dormitory. Slightly damp the forearm (or the calf) of the patient, and, turning the bottle right side up, let the patient's arm rest upon the mouth of the bottle (Fig. 28A).

In from a few minutes to half-an-hour or more the bottle will be noticed to have splashes of blood upon the bottom and sides (in the case of *Anopheles* only). If possible wait till all the *Anopheles* have fed.

Remove the bottle, invert upon a table, untie the twine, and remove the netting. Replace the bottle upon the prepared stopper.

Repeat the process every night, allowing the mosquitoes to feed by preference on the same case throughout. Where it is uncertain which of several cases may or may not have mature sexual forms in the blood, a bottle may be fed on alternate nights on the different cases.

Add clean water and a fresh piece of paper each time the bottle is used.

To Prepare Fed Mosquitoes for Dissection

After having fed the mosquitoes in a bottle for a certain number of days, place it apart from others, and allow it to remain undisturbed (merely changing the water, etc.) for several days.

Ascertain each day whether the mosquitoes have completely got rid of the blood in the midgut. When they are quite free from any dark colouration of the ventral aspect of the abdomen they are ready for dissection.

N.B.—If chloroform, and especially if tobacco smoke is used to kill the mosquito, it is essential to well wash the jars before again keeping mosquitoes in them.

To Feed Mosquitoes on Birds, etc.

1. Prepare a framework of wood and bookbinder's cardboard. Cover two sides with cardboard. Cover one end with netting drawn tight, and to the other attach a 'sleeve' of netting. Catch or breed out a number of *Culex* (*e.g.*, *Culex fatigans*), and place in the frame. Keep the frame in a dark place, and place a saucer of water in it.

Before placing the bird in the cage, a small *bag of netting should* be tied around its head, as it then remains perfectly quiet, and further, the legs *may* be *fastened*. Small birds, such as sparrows, should be carefully treated, as, otherwise, they are very liable to succumb. Pigeons should be treated in the same way, if necessary.

2. Mosquitoes *may* be *fed* singly on pigeons and other large birds by placing the end of the test *tube, in which the mosquito is confined*, against an area of skin denuded of feathers.

Feeding Experiments on Birds

1. Feed a number of *Culex*, *e.g.*, *C. fatigans*, on sparrows (in which have been detected proteosoma in the blood), by placing these for a time in the mosquito cage.

After feeding one or two days, place those mosquitoes, which obviously have fed and are gorged with blood, in a prepared chutney jar, and keep until ready for dissection.

Note (i) the zygotes of proteosoma which generally occur in large numbers in the stomach wall, and in which very coarse and *dark pigment* is seen.

(ii) *Feed some Anopheles on proteosoma sparrows*, and note that no zygotes are formed.

(iii) *Feed some Taeniorhyncus* on proteosoma sparrows, and note the negative result.

(iv) *Feed some Culex* upon pigeons containing halteridium, and note negative result.

Sparrows containing halteridium so frequently (in India) contain proteosoma that, even if the latter is not observed under the microscope, it is difficult to be sure of their absence.

Chapter XI

DISSECTION AND EXAMINATION OF MOSQUITOES FOR THE MALARIAL PARASITE

Dissection of Mosquitoes

Necessary Apparatus.

1. Slides and coverglasses

2. Two needles, preferably the straight surgical needles described for making blood films, as they have a cutting edge

3. Some salt solution, 0 5 grammes per cent.

4. It is convenient to have a board, twelve by three inches, covered half with white and half with black paper.

Some mosquitoes are caught by slipping over the top of the jar used for feeding another empty jar of the same size, and they may be kept alive in a dark cupboard for two or three days, until their stomachs are quite free from blood (seen by the complete disappearance of black from the ventral portion of the abdomen)

A few specimens are killed by chloroform or tobacco smoke.

1. Observe (if a gravid female) two whitish areas on either side of the hinder portion of the abdomen (ripening ovaries). If the blood in the stomach be not digested, a dark mass will be seen

in front of these, and possibly the extreme anterior portion of the abdomen will appear transparent (air containing *oesophageal diverticulum*). (Fig. 29.)

To Dissect Out the Mid-Gut (Stomach)

1. Pull off, with forceps, the legs and wings (and remove most of the scales by a few strokes of a small camel hair brush).

2. Place a drop of salt solution on a slide, and place the slide on a light background.

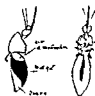


Fig. 29

Turn the mosquito upon its back and with a needle, held in the left hand, transfix the thorax.

Carry *the mosquito transfixed on the needle* to the slide, and lower the tip of the abdomen into the drop of salt solution.

Keeping the transfixing needle in position, make with the other needle, a nick upon either side between the sixth and seventh abdominal segments, which point corresponds to the division between the *mid and hind-guts*. After thus loosening the last few segments, place the point of the needle upon them, slowly dragging them away from the rest of the abdomen.

3. After separating the segments a very short distance, remove the preparation to a dark background. Again draw apart and note the white viscera stretching between them. Make

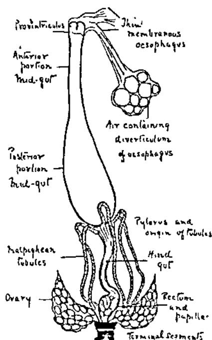


Fig. 30. Dissection of the Viscera of a Mosquito

steady traction until a central, rather transparent body is alone left between the two portions of abdomen.

Cut across the anterior attachments of the mid-gut.

4. Draw the body of the mosquito away from the separated segments; the mid-gut and sundry other viscera will be left attached to the latter floating in the salt solution.

Observe that when the tension is relieved, the structure last to leave the abdomen of the mosquito assumes a saccular appearance. This is the mid-gut.

The Viscera

(Fig. 30)

1. Unless the mosquito is newly hatched, note two opaque white oval bodies (the ovaries) attached to the separated segments. If the ovaries are near maturity, masses of white ova are seen.

2. *The Mid-gut.*—This extends from the level of the first pair of legs to the posterior border of the sixth abdominal segment.

(i) *An anterior narrow portion resembling an oesophagus.*

(ii) *A posterior dilated portion at the level of the sixth (and fifth) abdominal segments in which, if the last meal of blood is not quite digested, a black mass will be seen.* If any blood *remains in this portion, i.e.,* 'the stomach,' discard the specimen for one kept longer without food.

(iii) At the commencement of the mid-gut *a ring-like, thickened portion (the proventriculus).* It acts as a valve between the oesophagus and mid-gut (*Fig. 30*).

3. Passing between the mid-gut and the separated segments, *note five brilliantly white threads*—the malpighian tubules (Fig 30).

4. Between the malpighian tubules the transparent intestine which may exhibit active peristalsis (Fig. 30).

5. Attached to the proventriculus an exceedingly delicate membrane, the dilated oesophagus and three diverticula of the same, which usually contain air bubbles. They contain blood after a full meal, and, according to NUTTALL and SHIPLEY, these diverticula function as food *reservoirs*.

The ventral diverticulum extends as far back as the fifth abdominal segment.

TO PREPARE THE MID-GUT FOR EXAMINATION

1. Cut (by pressing with the needle) across the intestine and malpighian tubes just below the termination of the saccular mid-gut. This will separate the mid-gut from the rest of the viscera.

Remove everything from the slide but the mid-gut. Remove excess of fluid, and see that no ova or extraneous matters are left upon the slide. Add a small drop of clean salt solution, and place a thin coverglass upon the preparation. The mid-gut will flatten out considerably. Remove with filter paper applied to the edge of the coverglass any excess of fluid. Examine under one-third inch objective and afterwards under one-twelfth.

If the mid-gut has been removed *in toto*, and the preparation not too much compressed, the following appearances are seen :—

1. The narrow anterior portion of the mid-gut, with the calyx like proventriculus at its free end.

2. If a portion of the extremely thin membrane of the true oesophagus or its diverticula be included in the preparation, it will probably be seen to exhibit peculiar markings, due probably to muscular fibres in the membrane, but resembling rather closely sporozoits. It is essential that this structure should be recognized when seen, and that the resemblance of its markings to sporozoits should not lead the beginner astray (Fig. 32).

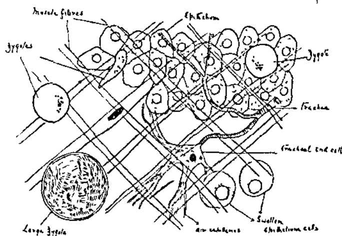


Fig 31 Microscopic appearance of Mid-gut, shewing Cell Structure and Zygotes

3. The expanded posterior portion of the mid-gut. This body forms the main mass of the preparation, and is all important in relation to malarial studies.

The following appearances are seen in a good preparation :—

1. Well-defined tubes with spiral lining (air tubes or tracheae). Note that these branch and

ramify upon the surface of the mid-gut and malpighian tubes (Fig. 31).

2. Large muscular fibres, together with elastic fibres, forming a check pattern. Note that they are circular and longitudinal (external). Note that at the edge of the viscus they are seen in optical section (Fig. 31).

3. Large cells with large nuclei and granular protoplasm (epithelium of mid-gut). Note some *in situ* forming a single layer of polygonal epithelium, and others detached and in process of being carried along by fluid streaming from interior of mid-gut. Note that in some places these cells are undergoing vacuolization with dancing of the protoplasm granules (Fig 31).

4. Note any contents of the stomach—
   - (i) Remains of blood.
   - (ii) Crystals of various kinds.
   - (iii) Gregarines, flagellates, bacteria, etc.

5. Note that in focussing downwards one passes through a double thickness of wall. Note that the air tubes are focussed on the upper and lower surfaces of the preparation, and the epithelium and crystals in the middle.

6 Trace several of the finer air tubes to their apparent termination, and note that when they lose their spiral lining they are continued as very fine transparent tubules (air capillaries). Note that at the point of breaking up, one can generally make out large stellate cells (tracheal cells). (Fig. 31.)

7. Observe in some preparations, large oval cells of brownish colour lying upon the outer surface of the stomach. Note that they are rather

opaque, and contain a certain amount of diffus yellowish pigment. They are so called pericardia cells (see Fig. 32).

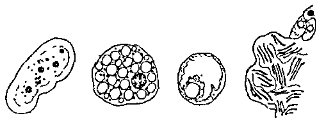


Fig 32 (Left to right) Pericardial Cell, Fat Body Cell, Swollen Epithelium Cell, Thin Membrane shewing Sporozoit-like appearance

8. Observe, in most preparations, one or more large clear cells with a small nucleus and filled with oil globules (cells of the fat body) (Fig 32.) These lie upon the stomach and, in common with the last named cells, are accidental in this situation.

The Examination of the Mid-Gut for the Zygote or Oocyst Stage of the Malarial Parasite

(The examination of the stomach blood for flagellating and the motile or vermicule forms is deferred to a later Chapter).

Obtain a number of *Anopheles* (not *M. Rossii*) from some native quarter (see p. 92), or better, those specially fed. Keep these alive for two or three days until no blood remains in the mid-gut (for methods of keeping alive, see p. 95).

Prepare the mid-gut as described above. A considerable number may prove negative, but a variable percentage will be positive. Examine with one-twelfth inch.

Carefully note the presence of small collections of pigment of the nature of *malarial pigment*. By careful focussing, the younger forms may be seen as clear oval or round bodies, 6-7 μ, in which the distinct and clearly defined pigment occurs. The more advanced forms can scarcely be missed. It is necessary to bear in mind the normal structures and the fact that, until the parasite reaches a considerable size and has a very sharply defined cyst wall, pigment, of the characters belonging to the species of parasite concerned, is present.



Fig. 33. (Left to Right) Zygotes of Malignant Tertian, Simple Tertian, Quartan, and Proteosoma.

1. Zygotes of crescent tertian shew, when young, a clump of pigment resembling black pepper grains (Fig. 33).

2. Zygotes of simple tertian shew yellowish or golden pigment in wisps (Fig. 33).

3. Zygotes of quartan shew rather coarse pigment in a clump (Fig. 33).

The older zygotes (40-60 μ) are indistinguishable as regards the species of parasite concerned. They may shew:

1. A very clear and distinct cyst wall (adventitious).

2. The formation of sporoblasts.

3. In still more developed forms, the sporoblasts are seen to be surrounded by a *radiating* arrangement of young sporozoits or blasts (Fig. 9 and 31).

4. Fully developed forms are large cysts packed with many hundreds of fine sickle-shaped bodies, and, if they are ruptured, these latter escape into the surrounding fluid, and are readily distinguished as sporozoits (Fig. 37).

To Make Permanent Preparations of Zygotes

Method 1.—In case of a specimen shewing zygotes, place a large drop of two per cent. formalin on one side of the coverglass, and draw this through by filter paper placed on the other side. Repeat several times. Remove excess of the formalin with filter paper. Ring edges of coverglass with black varnish or Canada balsam.

The zygotes will retain their appearance as seen in the fresh specimen.

Method 2.—Run formalin through as in Method 1. When an excess of fluid is present, slide off the coverglass. The flattened mid-gut will probably remain attached to the coverglass.

Wash in water, and stain lightly with methylene blue. Wash in water, and allow to dry. Warm gently to ensure complete dryness, and place the coverglass, mid-gut downwards, upon a drop of balsam upon a slide.

The muscular fibres and other structures of the mid-gut will be well exhibited. The zygotes will be stained rather a dark blue. If not too

darkly stained, the pigment of the zygotes will have the appearance it had in the fresh specimen.

3. Mount directly in glycerine.

4. For more minute histological examination, imbed the stomachs in paraffin or the whole mosquito (*vide* p. 123).

To Dissect Out the Salivary Glands

This is quite a simple proceeding if it be remembered where they lie. They are intra-thoracic structures, and they commence at the hinder portion of the neck and end opposite the first pair of legs. They lie ventrally, in fact, roughly speaking, they lie just above the origin of the first pair of legs (Fig. 34).

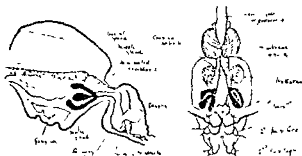


Fig. 34. *Showing position of Salivary Glands*

The simplest and most rapid method, and the one that hardly ever fails, is the following:

1. Place the mosquito in a drop of salt solution on its right side, with the head pointing towards you, as you dissect.

2. Place the needle of the left hand on the thorax to steady it, and place the needle of the right hand on the back of the head and make steady *gentle* traction.

3. If done carefully, it will be seen that the head has pulled out a little mass of white tissue from the thorax (the dissection is best done on a dull-black surface).

4. Examine the piece of tissue under a half-inch lens. (The diaphragm should be as nearly closed as possible). The glands will be seen hanging on to the neck as finger-like, transparent, *glistening* bodies. Muscle has a greyish look, and even the fat body is not so refractile as the glands.

5. Now place one needle on the head, and with the other make a transverse cut between the head and the attached portion of the glands.

6. Examine again the now separated glands. Generally all six are with certainty got in this way.

7. If the glands are not found on the neck, proceed with the dissection by Method 2.

8. When dissected out in this way, they are generally quite free from surrounding tissues, but if found necessary they can be teased out further *and placed in fresh drops of salt solution.*

9. At all stages of the dissection make sure that the glands are really present and that they have not floated to the side or stuck to the needles.

10. By this certain and rapid method, as many as one hundred glands may be dissected out, put under a coverglass, and examined microscopically in a day's work.

Method 2.—Consists in isolating, by a series of cuts, the anterior ventral portion of the thorax in which the glands lie.

1. Make a cut obliquely in an antero-posterior direction, so as to sever the main mass of thoracic muscle.

2. Make a cut at right angles to this, passing just behind the attachment of the first pair of legs.

3. Cut through the neck.

4. The glands lie in the portion thus isolated. Considerable teasing out is still required to isolate them from the surrounding tissues. Examine each portion of tissue separated out, and *remove to a fresh clean drop of salt solution.*

Remember that in examining under the microscope the apparent right hand is really the left, and *vice versâ.*



Fig. 35. The Salivary Glands of one side

This method, which is longer than Method 1, requires more dissection and teasing out in order to isolate the glands cleanly, and, as we have said, may still be followed, even if No. 1 has failed; but our experience has been that Method 1 is learned at once without any difficulty.

Ascertain that the glands of either side consist of three acini, the ducts of which join almost immediately after leaving the acinus to form a single long duct.

1. Observe that of the three glands of each side (Figs. 35 and 38) :—

(i) Two are highly refractile, and the cells in these are very distinct and clearly defined (lateral glands).

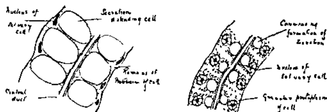


Fig. 36. Microscopic Structure of Salivary Acinus and that of a Newly-Hatched Mosquito (right)

(ii) One is much less refractile, and the component cells are much less easily defined (central gland).

2. Observe that each acinus has a duct running through its whole length, and that the secretory cells form a single row around this.

3. Observe that each secretory cell has a large mass of clear secretion within it, forming the chief bulk of the cell, and that the nucleus is flattened and pushed to the periphery (Fig. 36). Pressure tends to force the secretion out of the cell in viscid looking droplets. The secretion of the lateral glands is far more refractile than that of the central (Fig. 38).

4. Ascertain that the duct formed by the junction of the three intra-acinar ducts joins,

eventually, the similar duct from the other side, to form a common salivary duct which passes into the salivary receptacle. The duct is thick walled, and is lined with a spiral thread resembling a tracheal tube.

Examination of the Sporozoit Form of the Malarial Parasite

Obtain a number of *Anopheles* (not *M. Rossii*) from a native quarter (five per cent. to twenty per cent. or more have sporozoits in the glands), or *Anopheles* fed for twelve days or more at a temperature of 80° F. Prepare specimens of the glands as described above. Having placed one or more lobes under a low power, press with the point of a needle on the coverglass, so that the gland is ruptured, and the secretion poured out as droplets into the surrounding fluid.



Fig 37. Sporozoits in the Salivary Gland (Fresh preparation)

Examine with one-sixth inch. If sporozoits are present they are generally very numerous, and large numbers of fine, very distinct curved rods, will be easily seen with this power, lying throughout the fluid around the gland and packed in large numbers in the substance of the gland. Finally, examine with one-twelfth inch (Fig. 37).

The sporozoits have a mean length of 14 μ, and vary between 10 μ and 20 μ, and are 1-2 μ in width.

Examination of Motion of Sporozoits

Dissect out the glands and, when isolated cleanly, transfer to a drop of human serum, previously got ready by allowing blood to clot in a small tube. Three kinds of motion may be observed:—

1. Formation of curves.
2. Formation of ring-formed contractions.
3. Locomotion. Forward motion.

Penetration of Red Cell by Sporozoits.—This has not been seen in case of sporozoits of the salivary glands, but has been observed twice by Schaudinn in the case of sporozoits from a ruptured cyst in the stomach.

To Prepare Permanent Preparations of *Sporozoits*

Pressing firmly upon the coverglass, draw it along the slide, so that a film is made on coverglass and slide.

Dry by rapidly waving the slide and the coverglass in the air. Fix both in alcohol, and

stain with ROMANOWSKY. Wash, dry, and examine without coverglass with an oil immersion.

The sporozoits appear as fusiform bodies with a central red mass of chromatin. They are about $14\,\mu$ in length, with one end often more pointed than the other.

Wash off the oil with xylol, dry and label.

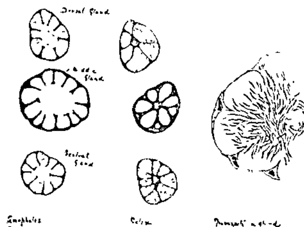


Fig. 38. Sections of Salivary Glands, showing differences between the Central and Lateral Glands and between those of Anopheles and Culex, also Sporozoits in the Glands of Anopheles

THE REPRODUCTIVE SYSTEM

To Examine the Spermatheca (Fig. 39).—By pressing with the edge of the triangular needle cut off the extreme tip of the abdomen - the last or eighth segment only. Place this in a very small drop of salt solution, and tease the fragment

carefully apart. A small black granule (sometimes two) will be seen. Isolate this as much as possible from other tissue and cover with a coverglass.

Observe, under a low power, a brown chitinous ball. Press firmly on the coverglass so as to rupture it. Examine under one-twelfth inch.

Observe the masses of fine hair-like actively motile bodies, if (as is probably the case) the mosquito has been fertilized. Isolate some of these; they possess the characters of spermatozoa (Fig. 39).

Examine the spermatheca of a mosquito newly hatched; it does not contain spermatozoa.

To Examine the Ovaries

Examine the ovaries of a number of mosquitoes caught in the room, etc.

Observe that when the ovaries nearly reach maturity they are readily detected as white areas on either side of the posterior part of the abdomen, and that when fully developed they occupy the whole of the lateral and dorsal portions of this.

Drag off the last few segments of the abdomen in a drop of salt solution, and allow the ovaries to float out in this. Observe that they are pyriform bodies, the apex being above (Fig. 39).

Ascertain that each ovary consists of a large number of follicular tubes, commencing as fine threads and ending in the oviduct. Observe especially the follicular tube forming the apex of the ovary, as here, this is most readily made out.

Ascertain that each follicular tube contains several egg follicles, the lowest of which is the most advanced in development.

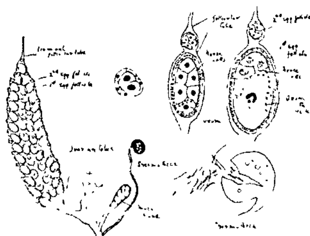


Fig 39. Spermatheca, with Spermatozoa Structure of Ovaries

Note that these latter show various stages of development in the different mosquitoes. Note that each consists of an outer layer of small cubical cells (follicular epithelium) and an inner mass composed of from four to eight large cells. Note the first appearance of the yelk as small oil globules in the lowest cell (the ovum), and how this increases until it nearly fills the follicle by encroaching on the other cells (nurse cells).

Observe in the mature ovum that the outer covering is formed of the original layer of outer cells (follicular epithelium).

The following organisms may be found in mosquitoes apart from the various stages of the malarial parasite :—

1. Encysted trematodes, mostly found in the tissues, near the neck.

2. Nematodes in the thorax or abdominal cavity.

3. Sporozoa. (*a*) Sausage-shaped bodies in masses, sometimes in close connexion with the salivary glands (Fig. 40).

(*b*) Octosporozoa, consisting of eight small sausage-shaped bodies in small cysts, enormous numbers of which replace the yelk of the ovum (Fig. 40).

(*c*) Flagellate bodies in the rectum and hind-gut, frequently in enormous numbers, and shewing, when stained, large numbers of developmental forms.



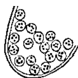






Fig. 40. Protozoa other than the Malarial Parasite found in Anopheles

(*d*) Gregarines. In the adult these have become encysted in the malpighian tubes. In the larvae, *the worm-like gregarine will be found* actively motile in the malpighian tubules.

4. The developing embryos of filariae. These are seen as sausage-shaped bodies with a terminal spike in the dissection of the salivary glands. In sections of mosquitoes they are seen in the muscles, especially in those of the thorax.

5. The developed embryos of filariae in the labium or about the base of the neck.

6. In the oesophageal diverticula masses of micro-organisms and sporozoa (?) will be found.

To Cut Sections of Mosquitoes

1. Kill some *Anopheles* by tobacco smoke or chloroform, or allow them to fall directly into absolute alcohol, by pouring a few drops into the tube containing them. Avoid, if possible, mosquitoes containing blood, as the blood becomes very hard.

2. Allow to remain in alcohol fifteen minutes to harden somewhat.

3. Remove one by one. Cut off with a fine scissors the legs and wings. Make a minute incision into both the thorax and abdomen, *e.g.*, holding the mosquito carefully between the finger and thumb, slice off with a sharp razor a portion of the dorsum of the thorax, and a minute portion of one side of the abdomen. This allows more perfect penetration of fluids.

4. Replace in absolute alcohol[1] (some authors recommend boiling in alcohol, as the air in the

1. For finer work pass through thirty, fifty, and seventy-five per cent to absolute. See, however, examination of separate organs.

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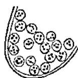






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tracheae is expelled, and the alcohol then penetrates completely), allow to remain one to two hours. This, which is to ensure complete dehydration, is the most important of operations, and upon it depends the success of the embedding. Make two or three changes, using alcohol to which $CuSo_4$ has been added.

5. Remove from alcohol and place for ten minutes or so in xylol. When the thorax becomes transparent, the mosquito should be removed, as too long a time in xylol produces much hardening.

6. Place for ten minutes in paraffin (*vide* p. 46) kept melted in a watch glass on the heated metal slab.

7. Smear a clean watch glass with a little glycerine, and fill with paraffin heated somewhat over melting point; transfer the specimen to this with a warm forceps to avoid cooling the paraffin.

8. Arrange the specimen as required. As soon as the surface of the paraffin has set, plunge the whole mass into water, as paraffin rapidly cooled is more homogenous.

If the watch glass has been smeared with glycerine it will be easy to remove the block.

9. Cut out the specimen, arrange as desired for cutting transversely, vertically, or horizontally. Take care that the top and bottom edges of the block are parallel. Smear these with a little melted soft paraffin (made by melting a little hard paraffin with vaseline). This gives an unbroken ribbon of sections.

To Mount Sections of Mosquitoes

Unless the sections are thick it is necessary to flatten them.

Heat some water in a dish a little below the melting point of the paraffin. Test the right temperature by dropping a section on the surface. If it melts, the water is too hot. If it does not flatten out, the water is not hot enough.

Drop upon the surface a ribbon of five or six sections; it will become perfectly flat if the temperature is right.

1. Smear some slides with solution of pyroxylon in oil of cloves (Appendix), taking care to avoid an excess. Lower the slide rapidly into the water, and with the aid of a needle draw it out again with the sections lying flat upon it.

2. Press lightly, but firmly, between smooth blotting paper. Protect from dust, and allow to dry for twenty-four hours, or dry more rapidly over the flame, but avoid melting the paraffin.

When dry, hold the slide a few seconds over the flame, this drives off the oil of cloves and melts the paraffin.

Pour over the slide first xylol, then alcohol, and finally place in water.

Mosquito tissues are so delicate that in mounting it is difficult to avoid the separation of portions or the whole of the section. This is especially so in the case of the chitin, which frequently breaks away.

OBREGGIA's method is recommended as giving *excellent results* :--

1. A slide is smeared with a thin film of a mixture, consisting of two parts of commercial liquid glucose and one part of a solution of dextrin (dextrin, 16 oz ; water, 17½ oz. ; thymol, 15 grains).

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Chapter XII

INTERNAL ANATOMY OF MOSQUITOES

THE ALIMENTARY CANAL

The alimentary canal is specialized on account of the blood-sucking habits of the mosquito. It differs from many insects in not possessing any caecal diverticula of the mid-gut. It also differs in the possession of five malpighian tubules, these being in insects usually even in number (Fig. 30).

The parts of the alimentary canals are as follows :—

The mouth The pharynx with pumping organ The oesophagus The oesophageal diverticula	The fore-gut.
The homologue of the proventriculus The stomach (so-called) The pylorus	The mid-gut.
The pyloric dilatation The small intestine The colon The rectum with rectal papillae	The hind-gut.

The mouth, pharynx, and oesophagus are ectodermal in origin, and both the mouth and pharynx are lined with chitin. The hind-gut is also ectodermal in origin; it does not possess, however, any portion lined with chitin. The

mid-gut is the true digestive portion of the tract.

The Pharynx.—The pharynx, which is lined throughout its extent with chitin, passes upwards and backwards through the ganglionic ring formed by the supra and infra-oesophageal ganglia and their commissures. At first it is narrow, but posteriorly becomes a large chamber (the pumping organ). (Fig. 43A).

The pumping organ occupies with its muscles a large portion of the head behind the level of the cerebral ganglia. In the state of rest its lumen is triradiate in transverse section. The walls are formed of three large and thick chitinous plates, one placed on either side and one superiorly. Into each of these plates powerful muscles are inserted. Those of the superior plate consist of two muscular masses, taking their origin from the occiput. Those of the lateral plates consist on each side of a single large muscular mass arising from the lateral portions of the head. The plates are connected by thin non-chitinous membrane, and their edges are rolled so that they form a spring capable of returning to their original position so soon as the separating force of the muscles ceases.

Posteriorly, where the pharynx becomes very narrow, a sharp bend occurs and a valvular action is produced. The whole forms a very powerful suctorial apparatus.

The Oesophagus.—Immediately beyond the pumping organ the chitinous layer ceases, and the rest of the fore gut is formed of excessively thin membrane. At the junction of the two portions a sharp bend occurs, and the floor projects so as to form a valvular flap.

The thin-walled oesophagus is a large dilated sac, whose walls are supported by surrounding structures. Into the posterior wall of the dilated and thin-walled oesophagus projects the papilla-like anterior portion of the mid-gut.

The Diverticula of the Oesophagus.—From the oesophagus two or three diverticula, similar in nature to the oesophagus, extend backwards. Of these, one is of great size, and usually contains air. This most usually extends into the abdomen, and is a prominent object in dissections and sections. In the newly-hatched mosquito it is small, but rapidly becomes large enough to extend into the abdomen (Fig. 30).

The Homologue of the Proventiculus.—There is no true proventriculus as in many insects. There is, however, an interesting fold of the fore-gut into the mid-gut which represents this organ. The anterior portion of the mid-gut has been noted as projecting into the dilated oesophageal pouch. This portion consists of both ectodermal and endodermal portions, and represents the proventriculus in other insects. The muscular bundles are here increased, and the whole forms a valvular muscular organ (Fig. 30).

The Mechanism of Feeding.—The powerful pumping action which must result from a drawing asunder of the three large chitinous plates of the pumping organ is very evident. These plates, also, when drawn apart must, by reason of their spring-like shape, revert to their original positions close together, without any muscular aid. Posteriorly the valve-like arrangement mentioned before prevents regurgitation.

The Mid-gut.—The mid-gut extends from the

proventriculus to the origin of the malpighian tubes. It consists of two portions which merge into one another—an anterior narrow portion, and a large dilated posterior portion, which becomes greatly distended after feeding. Unlike most insects there are no caecal appendages in the mosquito. Posteriorly there is a marked constriction, with strong muscular bundles, which forms a very marked pylorus (Fig. 30).

The anterior narrow portion of the mid-gut lies in the thorax, and does not become distended with blood. The posterior portion when fully dilated fills the greater portion of the abdomen, the viscera being pushed into the last few segments.

The Hind-gut.—The hind gut is short and passes in one or two bends from the pylorus to the anus. Immediately beyond the pylorus there is a considerable dilatation which is poorly supplied with muscular fibres: into this open the five malpighian tubules. For a short distance beyond this the lumen is narrow (small intestine), but becomes gradually larger (colon). At the termination of the colon there is a slight constriction, after which the canal dilates again to form the rectum (Fig. 30).

Into the rectum project six solid growths, the so-called rectal glands, which are, however, papillae. Posteriorly the rectum ends in the anus close above the gynaephoric canal.

The appendages of the alimentary canal are:—

The Salivary Glands.- The salivary glands consist of six tubular acini lying three upon either side. Those of one side lie generally one above the other in the long axis of the body, their

anterior ends lying close against the prosternum, where the ducts coming from each acinus unite to form a single duct. The upper and middle acini generally lie with their distal ends close to the proventriculus. The lower acinus passes towards the thoracic ganglion. Occasionally, an acinus becomes bifid at a short distance from its termination. A common abnormality also, is a small accessory acinus near the proximal end of an acinus. A duct can be seen traversing almost the entire length of each acinus. Shortly after leaving the acinus, the three unite to form a single duct. The duct of each side passes up into the neck, and lies close to the nerve cords passing between the thoracic and the cerebral ganglia. Beneath, and in contact with the lower surface of the suboesophageal ganglion, the ducts of each side unite to form a common salivary duct which passes forwards and enters the chitinous first portion of the alimentary canal close to the base of the proboscis (Fig. 34).

The Malpighian Tubules.—These are five in number and open into the first portion of the hind-gut immediately beyond the pylorus. Their blind ends are held in position in the neighbourhood of the rectum by tracheal branches. They pass *forwards in loops above their origin,* so that, in transverse section, as many as ten may be seen cut across.

The Muscular System

The chief muscular masses in the mosquito are contained in the thorax. They are chiefly muscles moving the wings and legs.

Wing Muscles.—There are two large muscular

masses on either side of the thorax, passing from the dorsal to the ventral body wall. Between these bundles there is a space, in the lower portion of which lies the alimentary canal, main air tubes, and other structures. The upper portion of the space is occupied by a second series of large muscular bundles, passing from the front to the back of the thorax. Neither of these large masses of muscle are inserted directly into the wings, the up and down movement of the wings being caused by alterations in the shape of the thorax, consequent on the contractions of the vertical and horizontal fibres, respectively.

There are, however, a few fibres arising from the lateral portions of the thorax, and inserted about the base of the wings.

Leg Muscles.—These occupy but little space in the thorax. They rise, to a large extent, from the internal processes of the exoskeleton (apodemes), and are inserted into neighbouring portions of the limbs. They arise also, from one segment of a limb and are inserted into another

The Muscles of the Body Segments.—These arise from one segment and are inserted into the next. They are arranged dorsally and ventrally in lateral groups throughout the abdomen.

A small muscle is also situated on each side, passing vertically from the tergum to the sternum. These on contracting flatten the abdomen.

Muscles in Association with the Alimentary Canal.—Several important muscular masses are connected with the large chitinous pumping organ. A pair of muscles arises from the occipital region of the exoskeleton, and is inserted into the upper plate of the organ. A large muscle arises on each side, and is inserted into each of the lateral plates.

Loop branches passing to the trunks, anterior and posterior.

Branches passing inwards and supplying viscera. Branches from the first, second, third, and fourth abdominal tracheae supply mainly the mid-gut, those from the fourth and fifth the ovaries, those from the sixth and seventh the genital organs.

The Vascular System.—As in most insects where the respiratory system ramifies throughout the whole body, the vascular system is not well developed. A dorsal vessel or heart and an anterior prolongation of this (aorta) are the only closed blood-vessels. Apart from the dorsal vessel the blood circulates in large blood spaces, which lie between the lobes of the fat-body and among the muscles and viscera.

The dorsal vessel passes close beneath the tergal plates throughout the abdomen. It is very thin walled, and is not provided with valves. The upper portion is attached to the dorsum at intervals by suspensory fibres (muscular), so that a festooned appearance is given in longitudinal section. There is, however, no true division into compartments. Laterally large cells (pericardial cells) are arranged throughout its entire extent, and fibres of a muscular nature (alary muscle) pass from the body wall and end in branches in close connexion with the dorsal vessel.

At the first abdominal segment the dorsal vessel dips down beneath the mesophragma, lying as it does so in direct contact with the cuticule. In the thorax it again arches upwards, and lies between the lower portions of the antero-posterior wing muscles close above the ante

In the anterior third of the thorax it divides into two smaller portions which pass outwards, and coming in contact with the salivary ducts enter the neck.

Blood spaces without definite walls occur throughout the body. The thorax especially contains large spaces among the muscles, and the complex fat-body which lies between and supports the organ is everywhere bathed with blood fluid.

The Nervous System

The ganglionic system in the *Culicidae* is considerably developed. The head ganglia are large and complex. The thoracic ganglia are large and compressed so as to form a large ganglionic mass. The ganglia of this system are as follows:—

(*a*) Lying around the pharynx is a ganglionic ring composed of large supra and infra oesophageal ganglia with their commissures. From these, large nerves go to the eyes, antennae, and mouth parts.

(*b*) In the thorax, lying below the oesophagael diverticulum and close to the sterna, is a large compound ganglion showing evidence of its origin from the conjoined ganglia. Between this and the head ganglia are two long slender nerve cords, which pass in the neck in close relation with the salivary ducts. From the thoracic ganglion large nerves pass to the limbs, and posteriorly nerve cords connect it with the first abdominal ganglion.

(c) The abdominal ganglia lie with their connecting commissures close upon the abdominal

sterna. The last ganglion lies just below the junction of the oviducts to form the common oviduct. A large nerve passes from it among the viscera of the last few segments.

The Visceral System.—Small ganglia connected with the main ganglionic system occur in connexion with the viscera. The most important of these are two small groups of large nerve cells lying in front of and above the thoracic ganglion, with the middle portion of which they are connected by nerves. They lie laterally beneath the oesophageal diverticulum and anterior portion of the mid-gut, and are not far removed from the salivary glands. Another small ganglion occurs above and in front of the proventriculus (Fig. 34).

The Reproductive System

The organs of the reproductive system are:—

1. Ovaries.
2. Oviducts and common oviduct.
3. Mucus gland and duct.
4. Spermathecae and ducts.

The ovaries occupy a variable position dependent upon the state of their development. In the newly-hatched mosquito they are small bodies lying in the fourth and fifth abdominal segments close by the posterior portion of the mid-gut, and attached to the body wall by numerous tracheae. As they enlarge they push the mid-gut, hind-gut, and malpighian tubes towards the ventrum, so that eventually the ovaries occupy nearly the whole of the posterior portion of the abdomen. Each ovary consists of very many follicular tubes, each containing egg follicles in different stages of

development. In the mature ovary the lower follicles have in every tube become the large completely-formed egg (Fig. 39).

The oviducts are muscular tubes passing from the ovaries. They join beneath the rectum to *form the common oviduct*, which is still more abundantly supplied with muscle fibres, and which eventually opens beneath the anus.

The spermatheca is a chitinous sac, which in the impregnated female is filled with a mass of spermatozoa. Its duct is long and twisted and opens into the common oviduct near its termination.

The mucus gland, globular or ovoid in shape, opens by a short duct into the same region

The Fat-body.—The adipose tissue is disposed in two ways.

1. As a general lining to the body wall, being nearly everywhere present directly beneath the cuticle, and

2. As lobular masses lying in among the organs and muscles. Thus a large pad lies over the compound thoracic ganglion, and sends processes which lie in among the salivary glands and other viscera. Other smaller masses lie in the head and abdomen.

Histology

Methods.—The examination of the fresh tissues frequently reveal structures not easily seen in fixed preparations. The tissues are best dissected out in normal saline of low tonicity, 0·3 or 0·4 per cent., as insect juices have a lower isotonic point than those of mammals. Better preparations of

a double thickness of mid-gut wall as well as the fore-gut. There is an increase in the muscular fibres of the mid-gut at this point, especially the circular fibres, so that a very distinct mass is formed homologous to the proventriculus of many insects. There is no chitinous development, however, and the structure would appear to act only as a muscular sphincter (Fig. 30).

The Hind-gut.—The nature of the epithelium and arrangement of the muscular fibres differs somewhat in different portions of the hind-gut. Structurally the small and large intestine are similar, whilst the dilatation beyond the pylorus, and especially the rectum, differs from these.

The dilatation which occurs at the origin of the malpighian tubules is thin-walled and poorly supplied with muscle fibres. The cells lining it are small and flattened.

The intestine is lined with a single layer of large cubical cells; external to these is a muscular coat. The cells of the intestine have large nuclei which have a similar, though more open, arrangement of the chromatin than the nuclei of the mid-gut. The protoplasm is finely reticular, and stains less deeply than the cells of the mid-gut. Stained with Heidenhein's haematoxylin, no granules are present as in the cells of the mid-gut. They have no striated border.

In the rectum the cells become small and flattened. There are here, however, bodies usually termed rectal glands. These are papillae covered with a single layer of much hypertrophied cells resembling those lining the small intestine and colon.

The muscular system of the hind-gut is very

similar to that of the mid-gut, consisting of very large fusiform, straited cells arranged circularly and longitudinally. The circular fibres in the small intestine lie outside the longitudinal, and pass spirally around the mid-gut. Towards the termination of the intestine longitudinal fibres also lie outside the circular. In the rectum and extending throughout the hind-gut and mid-gut in both *Anopheles* and *Culex*, there are, in a large proportion of specimens, swarms of a flagellate organism (Fig. 40).

The Salivary Glands - The salivary acini lie in a cleft in the fat body, which latter comes in close contact with the glands. Each gland acinus consists of a single layer of large cells, limited externally by a delicate sheath (basement membrane) and internally by the intra glandular duct wall.

In *Anopheles* the intra-glandular duct becomes larger as it approaches the termination of the acinus, and forms a large cavity.

In *Culex* the duct remains of the same diameter throughout the acinus, and terminates abruptly near the end of the acinus without any dilatation.

In both *Culex* and *Anopheles* there are two types of gland acinus. These are recognizable both in the fresh gland and in fixed specimens. From their appearance in the latter they may be termed

(1) The granular type

(2) The clear or colloid-like type.

The Granular Type- The greater portion of the acinus consists of cells whose nucleus and protoplasm has been pushed to the outer portion

of the cell by a large mass of secretion, which occupies almost the whole of the cell. In the fresh gland this secretion appears as a clear, refractile substance, and can, by pressure, be made to exude from the cell in refractile globules. In specimens hardened in alcohol, this clear secretion appears as a granular mass, occupying the greater portion of the cell. It stains faintly with haematein, and shows under high powers (one-sixteenth oil immersion) a coarse reticulum and isolated globules, an appearance probably due to the precipitation or coagulation of the secretion by the alcohol. Considerable variations exist, however, in the appearance of this granular secretion, both in the different mosquitoes and in different parts of the same gland. In *Anopheles* the greater portion of the gland contains cells densely crowded with granular material. Very frequently, however, the terminal portion contains cells in which only a few large globular masses exist (Fig. 38).

The protoplasm of the cell occupies, in the fully-matured gland, only the extreme periphery, and the nucleus, which is much degenerated, is pushed to the outer portion of the cell, and usually lies in the angular interval left at the base of two or more contiguous cells. In the granular type of gland this disappearance of the protoplasm and nucleus from view is more pronounced than in the clear type of gland.

The Clear or Colloid-like Type.—Of the last-mentioned type there are two acini upon either side; of the present type there is but a single acinus upon either side, which usually lies between the two acini of granular type (Fig. 38).

In the fresh gland the cell outlines are not so

distinct as in the granular type, and the secretion, when extended by pressure, is much less refractive. In alcohol-hardened specimens, the acinar cells contain a large mass of clear, homogenous secretion which, as in the last-mentioned type, fills almost the entire cell, and pushes the protoplasm and nucleus to the periphery.

In the clear type, however, the protoplasm is always in greater amount than is the case with the granular type, and the nucleus never becomes so greatly degenerated. The clear, homogeneous secretion stains readily with haematein, and may even stain quite deeply. With HEIDENHEIN'S haematoxylin it frequently becomes almost black. It resembles very much in appearance colloid substance as it is seen in the mammalian thyroid.

In *Anopheles* this substance also distends the central duct space within the acinus. In this situation an appearance is sometimes produced which resembles faintly-stained sporozoites, but which is a normal condition.

The Maturation of the Glands.—In freshly-hatched mosquitoes both types of acinus consist of large glandular cells arranged round the lumen. These contain a large, centrally situated nucleus, and have protoplasm containing a large number of coarse granules, staining with haematein. In the portion of the cell nearest the lumen a vacuole of varying size is situated. This is the commencement of the large mass of secretion which, in the mature gland, occupies the entire cell. In the granular type of acinus the vacuole contains granules; in the clear type it resembles the colloid-like secretion (Fig. 36).

Further Variations in the Cells of the Salivary

Acini.—In the granular type of gland the greater portion of the acinus is composed of cells of the character described above. A portion, however, usually exists which differs considerably in structure. This portion adjoins the duct, and may, in *Anopheles*, reach as much as one-quarter of the entire gland in length. In this portion of the gland the cells are much smaller than those containing the granular secretion, so that the diameter of the acinus is much less here, and a sudden increase takes place when the portion containing the granular secretion is reached. The cells lying towards the duct differ from those lying towards the acinar end of this portion. There is, however, no line of demarcation between them, the one gradually becoming changed into the other. In the centre of each cell is a clear body, pushing the nucleus and protoplasm to the outer portion of the cell. Towards the duct end in the centre of this clear substance is a darker portion continuous with the duct lumen. As the cells come to lie nearer the distal portion, this central dark lumen becomes obliterated. This structure, though present in *Anopheles*, may be absent in *Culex*. In certain *Culex* another variation in the gland cells frequently occurs. The portion of the gland lying close to the duct, instead of being less in diameter is greater. The cells composing this portion are columnar in shape, with centrally situated nuclei and no contained secretion.

In certain specimens it is not uncommon to find cells occupying a peripheral position, and not approaching the lumen, which contain a substance resembling the colloid-like secretion of the clear type of gland.

Changes after Feeding.—Very little change occurs in the glands after feeding. They are for the most part still quite full of secretion. Probably a very small amount only of secretion is used with each puncture.

The Ducts.—The intra-acinar ducts vary in *Culex* and *Anopheles*. In *Culex* they remain narrow and tubular throughout the entire length of the gland. In *Anopheles* they become large spaces in both types of acini, but especially in the clear type. The duct is lined throughout by a clear homogeneous skeletal material, which is continuous with a similar substance dividing the cells of the gland from one another. Into the duct the secretion-filled cell opens by means of a small opening.

The duct, after leaving the acinus, consists of a thick-walled tube, with a central spiral thread resembling the spirals in the trachea. The wall is homogeneous, but contains many nuclei.

The Malpighian Tubules.—The malpighian tubules are tubular bodies with caecal ends, which open into the hind-gut. The cells are extremely large, being, next to the pericardial cells, the largest in the body. Each cell contains a large nucleus, and contains numerous large granules, which stain feebly with haematein, but powerfully with HEIDENHAIN's haematoxylin. Numerous fatty granules are also present. Each cell is wrapped round a central lumen, the cells being arranged alternately, so that a zig-zag appearance is given in section. The inner portion of each cell is markedly striated, the lumen being thus bounded by a striated area. In relation with these tubules, a large number of tracheae and tracheal end-cells exist.

In certain conditions the malpighian tubule cells may be found quite free from granules, though otherwise unchanged. This change occurs in mosquitoes with large numbers of a flagellate organism (previously noted) in the rectum and hind-gut.

The Muscular System.—The muscular fibres of the mosquito are without exception striated. Those of the wings differ in structure very much from those of the limbs and body segments. The muscle fibres of the alimentary canal are large fusiform cells, with a single large nucleus with some surrounding protoplasm. The muscle fibres in connexion with the heart are much branched.

Many of the fibres contain a very marked sarcolemma and space between this latter and the fibre. This space is usually seen occupied by extremely delicate branching threads, which stain feebly with haematein.

In the pupae there exist some large cells of peculiar nature in association with the sheaths of the muscle fibres.

The structure of insect muscle is described in many works on histology, and does not need repetition here.

The Tracheal System.—The larger tracheal vessels consist of a single layer of flattened cells with an inner chitinous layer. In smaller tubes the cells embrace the entire vessel, the nucleus frequently being bent around the lumen. The cells of the tracheal vessels contain numerous small clear vacuoles (chitin formation). The chitinous lining possesses a thickening in the form of a spiral thread, which may become unwound and lie stretched as a wavy thread in fresh preparations.

The smaller tubes contain the spiral thread until they become from 2 to 5 μ in diameter. They then divide to form bundles of excessively minute air capillaries, which enter among the tissue cells. The division into capillaries takes *place in the substance of large branched* cells situated at the termination of the tracheal vessels. The cells often appear cribriform in section from the number of air capillaries. These cribriform cells in connexion with the tracheal endings are well seen in the mid-gut and malpighian tubules. *They are, however, seen best of all in the* undeveloped ovary of the newly-hatched mosquito, which is extremely rich in bundles of capillary air tubes.

The Vascular System.—The dorsal vessel is a delicate walled tube composed of longitudinal and oblique fibres with a nucleated inner layer. The fibres may be traced directly from the terminations of the branched alary muscle fibres. The alary fibres break up into fibres which pass in close connexion with the large pericardial cells, and *eventually form (1) fibres passing into the dorsal* vessel as longitudinal fibres, (2) fibres joining in an anastomosis in connexion with the floor of the dorsal vessel.

The pericardial cells are extremely large cells lying on *either side of the dorsal vessel* throughout its whole extent. They are by far the largest cells in the mosquito, varying from 30 μ to 50 μ in longitudinal diameter. They are elongate or pear-shape in form, and contain several nuclei. The nuclei usually show signs of degeneration. The peripheral portion of the cell stains *more deeply* than the central portion, which contains the

nuclei and small stained granules. There are a considerable number of masses of a light yellowish pigment resembling that found in the large visceral ganglia cells. The fibres from the branches of the alary muscles pass over and around the pericardial cells to reach the dorsal vessel. From their structure and situation the pericardial cells appear to be of the nature of ganglion cells (Fig. 32).

The Fat-body.—The fat-body, both where it occurs as a portion of the body wall and where it lies as free lobulated masses, consists of cells containing numerous oil globules. The cells are of considerable size, and their borders may be frequently traced as polygonal areas. The nuclei are oval in shape with a central mass of chromatin and chromatin threads. Besides oil globules the cells contain granules staining with haematein, and minute droplets of a highly refractile, dark substance, which gives the appearance of pigment. These droplets are larger in amount in old mosquitoes than in those freshly hatched (Fig. 32).

The Nervous System.—The ganglia of the ganglionic system consist of an outer portion of nerve cells and an inner portion of non-medullated nerve fibres. Considerable complexity exists in the larger ganglia, especially the head ganglia.

The ganglia of the visceral system differ greatly from those of the ganglionic system. The ganglion cells are few in number and of large size. They possess clear reticular protoplasm, a little denser around the periphery than in the centre. Around the inner margin of the denser peripheral portion small stained points are arranged. In the centre a variable number of granules of yellowish pigment exist.

The Reproductive System.—Each ovary consists of a large number of follicular tubes whose lower ends open into the ovarian tube, and whose upper ends terminate in a delicate supporting filament (terminal filament). The apex of the ovary is formed of a single follicular tube, whose filament is attached to the fat-body of the fourth segment.

Around the whole ovary there is a delicate *nucleated sheath.*

Each follicular tube contains one or more *egg-follicles in different stages of development.* In the freshly-hatched mosquito each follicular tube contains an undeveloped egg-follicle. As this develops, a second and a third undeveloped follicle appear above it, which again undergo development into mature eggs. The follicle at first consists of two to four large cells, with large nuclei surrounded by a single layer of smaller epithelial cells (Fig. 39).

The central cells then increase in size and number, so that many very large cells are contained in the now enlarged follicle. The surrounding epithelial cells also become larger, and rapidly increase in number so as to form a layer of regular cubical cells surrounding the follicle. The central cell nearest the ovarian tube is the ovum, the rest are nurse cells, and eventually disappear. *Both the ovum and the nurse cells* increase greatly in size. The nurse cells have clear *protoplasm and extremely large nuclei,* which exhibit karyokinetic figures. The ovum contains very numerous yolk granules, which occupy the whole of its substance, except a thin coating of granular protoplasm. Still later this thin

external layer can only with difficulty be made out (Fig. 39).

The nucleus of the ovum undergoes very pronounced changes. It appears as an irregular mass, staining uniformly with nuclear stains. This mass becomes more and more distorted and broken up, and eventually disappears. It may frequently, however, be seen as irregular masses of staining material even in the mature egg. A portion of the nucleus is seen very early to be separated off from the rest, often surrounded by the latter. This portion (female pronucleus) is small and difficult to detect in sections in the more mature ovum. As the ovum increases still more rapidly in bulk, the nurse cells become crowded into the distal portion of the follicle and eventually disappear, so that, in the mature egg, no trace of them is to be seen. The epithelial layer surrounding the follicle becomes much flattened, and forms eventually a covering to the egg (chorion). The outer portion of this covering (exochorion) is transparent, and marked with oblique parallel markings. Over the proximal end, *i.e.*, the end lying towards the ovarian tube, the chorion forms a globular mass ornamented with rows of pits. This is the micropylar apparatus through which the spermatozoa penetrate the ovum.

Frequently in *Anopheles* a large portion or the whole of the adult ovum consists of a mass of sporozoa. These consist of numerous small cysts, each containing eight round or crescent-shaped bodies, each with a central chromatin spot (Fig. 40).

The ovarian tube arises in the centre of the ovary, and receives on all sides the follicular

ibes. It is lined with a single layer of small
ibical epithelium. After passing out of the
vary, a considerable number of striated muscular
ores are arranged in a loose network around it,
nd pass from it to surrounding structures. There
re also muscular fibres in the ovary itself in con-
exion with the ovarian tube and egg-follicles.

The spermatheca consists of a chitinous sac,
ith large cells lying externally. These resemble
ie cells of the cuticle, and contain droplets.
hey do not cover the whole of the surface of the
permatheca. The contents of the spermatheca
i the fertilized insect consist of a mass of sper-
iatozoa, which, in the fresh state, may be seen
evolving with great rapidity within the sac. The
permatozoa have a narrow, slightly-curved head
nd a long tail. The duct of the spermatheca is
arrow and thick-walled, and contains muscular
ibres. Certain large cells lie in connexion with
he duct externally. The mucus gland contains
ells filled with secretion. There are small nuclei
n connexion with the intra-acinar duct (Fig. 39).

Chapter XIII

TO COLLECT AND PRESERVE MOSQUITOES

How to Collect Mosquitoes

Mosquitoes may be collected in two way:—

1. By capturing the adult flies.
2. By breeding out from larvae and nymphae.

1. Search in the daytime in houses, huts, and out-houses, at the base of large trees, amidst brushwood, and other dark or shaded places. *Anopheles*, however, are rarely caught except in huts and out-houses. They are especially fond of cow-sheds and the darker portions of the eaves of huts.

Some species of mosquitoes may be caught by sitting with a light near a white wall or suspended sheet, or inside a tent, near a jungle or marsh. *Culex* and *Taeniorhyncus* may be found sitting on the surface *just beyond* the brightly illuminated area. *Anopheles* are rarely caught in this way, but, one species at least *(A. barbirostris)*, appears to be attracted by light, and was caught by us on an illuminated sheet at night, near swampy land.

In searching for adult *Anopheles*, as many places as possible should be examined, as the distribution of some species is very local.

If the captured insects appear to have fully matured ovaries, some of these should be placed in bottles, as previously described (p. 97), and allowed to lay their eggs.

If care is taken to place only one species in a bottle, the characters of the ovum may be noted, in addition to the adult insect

Some of the ova should be placed in fresh water, and an attempt made to determine the characters of the larva (p 73), when it has hatched out and is sufficiently grown.

2. *Breeding out*.—Full grown larvae, and especially nymphae, are collected. These are collected from every possible source. Scarcely any water will be found free from some form of mosquito larvae. Even strongly brackish waters, containing over one per cent. of salt, often contain large numbers.

Examine water from the following sources:—

(i) Domestic utensils, cisterns, tins, pots, calabashes, in which there has been water for three or four days. The larvae of *Stegomyia*, *Culex*, etc., and only rarely *Anopheles*, will be found.

(ii) Cess pits, pools full of decaying leaves, etc., sewage ditches. Note larvae of certain species of *Culex*, etc.

(iii) Observe presence of the larvae of *Stegomyia* and *Culex* in the water which collects in the axils of banana leaves and other plants. Also, occasionally, *Anopheles* in large collections of water of this kind.

(iv) Puddles of all kinds, with and without algae, ponds, tanks, swamps, rice fields, ditches, canals, rivers, streams, lake margins, and wells,

scales are rubbed off, and that a crookedly mounted specimen is better than a 'rubbed' one.

5. Push the pin steadily through the thorax, so that it emerges as near the centre of the dorsum of the thorax as possible. [Practise mounting by forcing the fine pin through without aid from the other hand.]

6. Having transfixed the mosquito, force the point of the pin one millimetre beyond the back, by pressing it against the smooth surface of a cork or tissue paper. The pin should not be pushed through too far, as it prevents the lens of the microscope being brought near enough for examination.



Fig 41. Authors' Method of Preserving Mosquitoes

7. Placing the disk against a cork, pass carefully through the edge a large entomological pin. This is passed in the reverse direction to the fine pin. Force three-quarters of the length

of the large pin through the cardboard disk, and then firmly press the point into the cork of a specimen tube, so that when the tube is corked the mosquito is inside (Fig. 41).

In damp climates, it may be necessary to carefully dry the tube and insect in a dessicator (over sulphuric acid or lime), or by placing in the sun or warm place to prevent mould. This, however, is but seldom required. Mites are rarely seen in insects preserved in tubes as described.

Write any information, *e.g.*, locality, date or reference number *upon the outer surface of the cork* and on the edge of the cardboard disc. For transmission, all that is necessary is to pack the tubes in wool in a box and send by post. Packed in this way they are far more secure than when mounted in the ordinary way in an entomological box. Mosquitoes for the British Museum should be addressed :

The Director
The British Museum
(Natural History)
Cromwell Road, London, S.W.

Endeavour always to send both male and female, at least two of each, and, what is of the greatest possible importance for the advance of our knowledge of mosquito classification, the careful description of ova and larvae.

Note. If from any cause it is impossible to pin and mount mosquitoes in this way, they may instead be simply placed between layers of tissue paper in a pill box, etc. This is far better than placing them in any fluid such as spirit, by which treatment they are rendered useless for identification.

To Mount Portions of Mosquitoes Permanently

1.—*Wings.*—Clip off one or both wings as near as possible to the thorax, so as to avoid cutting the base of the wing itself.

2. Allow the severed wing to fall in the centre of a clean glass slide. See that the dorsal surface of the wing is upwards.

3. Place one or two very minute drops of *thick* Canada balsam at some distance from the wing, but within the area of a coverglass. This is merely to hold the coverglass firmly in place; the balsam must not be allowed to touch the wing.

4. Press a coverglass firmly down on the Canada balsam. If it is desired, the coverglass may be ringed with paraffin or some material which will not run by capillarity beneath the coverglass.

Legs.—Mount the legs of one side in order in a similar way.

Mount the palps and proboscis.

Mount (*a*) The male ungues.
(*b*) Scales from head, scutellum, etc.
(*c*) Wing denuded of scales.

In Canada balsam, by placing a drop of balsam on these and mounting in the ordinary way.

Chapter XIV

ANOPHELES. EXTERNAL ANATOMY OF THE IMAGO

The Head

The head is composed mainly of the two large compound eyes. These meet below and approach one another very closely above.

Parts of the head. The following are the usual names for the different regions of the head (Fig. 42).

1. The nape: the extreme back of the head
2. The occiput: the portion behind the eyes
3. The vertex: the space between the eyes.
4. The frons: the space in front of the eyes
5. The gena: the side of the head below the eyes

The frons is triangular in shape, with one angle directed downwards. From the upper two angles arise the antennae, and from the lower projects the clypeus, lying over the base of the proboscis.

The Clypeus. Projects over the base of the proboscis as a prolongation of the frons. The character of the clypeus is of specific importance. It is

1. Hairy in *Culex*.
2. Scaly in *Stegomyia*.
3. Nude in *Joblotia*.

The Antennae.—Consists of fourteen to sixteen segments, of which the basal one is large and globular. The plumose antennae of the *male* readily distinguishes it from the female.

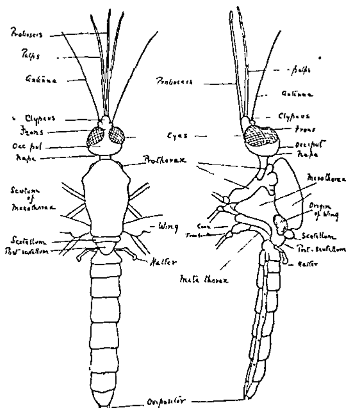


Fig. 42 External Anatomy of Female Anopheles

The Proboscis.—The proboscis consists of the very highly specialized mouth parts, ensheathed

in the lower lip or labium. The proboscis consists of (Fig. 43) :—

1. The labium, forming the sheath.
2. The labrum and epipharynx, or upper lip } forming the *stylets.*
3. The hypopharynx, or tongue } forming the *stylets.*
4. *Two mandibles* } forming the *stylets.*
5. Two maxillae } forming the *stylets.*
6. Two maxillary palps.

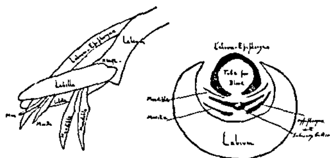


Fig. 43 The Proboscis (Labium and Stylets) after NUTTALL *and* SHIPLEY *Right hand, cross section of Proboscis The Palps are not shown*

The Labium.—The labium forms the thick and scaly proboscis as usually seen. On its dorsal surface it is hollowed out, and in this hollow run, as in a sheath, the piercing mouth parts or stylets (Fig. 43). The labium itself does not penetrate the skin, but becomes sharply bent during the act of biting, just as when a cane walking stick is pushed against the ground. This may easily be seen if a mosquito is watched during the process of biting.

The Labellae.—Attached to the end of the labium by a hinge joint on either side are two leaf-like processes, the labellae (Fig. 43). It is through the angle made by the two labellae, that the stylets pass, as a billiard cue, between the first and second fingers (NUTTALL and SHIPLEY).

The labium proper stops short at the point of junction of the labellae, but is continued on its upper surface as a blunt point covered with fine hairs (DUTTON). We may liken it to a pen continued on beyond the penholder, the junction of pen and penholder being the point at which the labellae are hinged on.

Dutton's Membrane.—The area between the end of the labium proper and the extreme tip is covered by an extremely thin membrane (DUTTON). In the act of biting, when the labellae are separated, this membrane is somewhat stretched, and applied to the skin.

THE ESCAPE OF THE FILARIAL EMBRYO

It has been shewn by LOW and JAMES that the filarial embryo occurred in the proboscis, according to LOW among the stylets. According to DUTTON, the embryo lies really in the tissue of the fleshy labium, moreover with its head at the level of the membrane described above, and that it is by the rupture of this excessively thin membrane that the embryo escapes. GRASSI and NOE think that the embryo escapes through the middle of the bent-up labium through a rupture at this point, but DUTTON's explanation seems more likely.

The *epipharynx* is the central tube through which the blood is sucked. Its point slopes off

somewhat like the tip of a hypodermic needle. In cross section it has the shape of an Ω, the completion of the tube being formed by the apposition below of the hypopharynx. The labrum is blended with the epipharynx, but does not extend to the tip.

The hypopharynx is a thin, flat two-edged lamella closely applied to the under surface of the epipharynx. It is pierced by the salivary duct down which the salivary secretion and sporozoits pass. The opening of the duct is continued as a groove reaching almost to the tip of the hypopharynx.

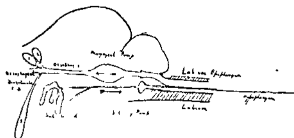


Fig. 43A. Showing relation of Pharyngeal and Salivary Pumps to the Proboscis

The *mandibles* are very fine chitinous rods in cross section, crescentic in shape. At the tip of the mandibles are about thirty serrations, though in certain species of *Culex* these appear to be absent.

The mandibles are closely applied to the sides of the epipharynx.

The *maxillae* are stouter than the mandibles, and fit around the outer side of these and the

hypopharynx. They have about twelve serrations at the extremity, coarser than those of the mandibles. In some culices, papillae replace the serrations.

The Maxillary Palps.—These lie upon either side and somewhat dorsally to the proboscis. In the act of biting they take no part, but are then separated from and lie at right angles to the proboscis. Differences in the palpi are of both specific and generic importance.

The expanded ends of the palpi in the male *Anopheles* are even more conspicuous than the plumose antennae.

The Prothorax.—The main portion of the thorax is mesothoracic; anteriorly, however, there is a collar-like piece of chitin, the prothorax. To this are attached two moveable bodies, the patagia.

The prothorax is of importance in classification, *e.g.*, in the new genus of the *Anophelina Stethomyia* the prothoracic lobes are mammillated.

The Mesothorax (Fig. 42).—The *scutum* of the mesothorax forms the large globular mass of the thorax. Behind the scutum, and just behind the origin of the wings, is a transverse bar of chitin, the *scutellum*. Behind the scutellum is a convex triangular area extending as far as the first abdominal segment, the *post-scutellum* (Fig. 42).

The scutellum and post-scutellum are of importance in classification. Thus the scutellum, with its 'posterior border bristles,' is often of specific value, whilst the post-scullem may be—

1. Bare. *Culex* and *Anopheles.*
2. With hairs. *Wyeomyia.*
3. With scales and hairs. *Joblotia.*

THE WING

The wings shew :—

1. An anterior straight, thick, and strong border or costa.

2. A posterior curved and thin border, carrying a fringe.

3. Two small folds at the base of the wing (squama and alula).

4. Nervures, or veins.

The *costa* in *Anopheles* is generally covered in part with white, and in part with black, scales (spotted).

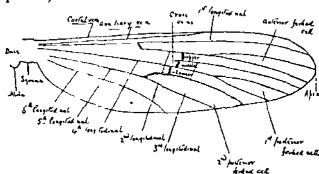


Fig 44. *Wing of Anophelina* —
Upper = Supernumary cross-vein
Lower = Posterior cross-vein
Anterior forked cell = First forked cell
First posterior forked cell = Second posterior cell
Second posterior forked cell = Anal cell

The *fringe* in *Anopheles* has most frequently light and darker portions, the number and position of which are of specific importance (Fig. 51).

The *squama* and *alula* are shewn in the figure. They are of no great importance (Fig. 44).

The Nervures.—The nervuration of the wing is of considerable importance. Several nomenclatures are in use. That used in the accompanying diagram is, however, the simplest (Fig. 44).

In classification, the relative position of the apices of the two forked cells are frequently used. Also the relative positions of the point where the auxiliary vein cuts the costal vein, and the point where the fifth vein cuts the posterior margin. As a rule, the position of this first point is much nearer the base than that of the second point, but in a few instances, *e.g.*, *M. sinensis*, they almost coincide.

Also the positions of the upper, middle, and cross veins. It will be found, however, that even in the same species there is no constancy in these latter, and they can hardly be given as of specific importance as has been done. DÖNITZ has made the same criticism, and indeed, finds that the position in each wing of the same mosquito may be different.

THE LEGS

These consist of the following segments:—

1. Coxa and trochanter. Small pieces at the origin of the legs (Fig. 42).
2. Femur.
3. Tibia.
4. Tarsus, consisting of five segments, the last of which carries the claw or ungues.



Fig. 45. *Fore Ungues of M Funesta (♂) the larger Uniserrate. Fore Ungues of M. Rossii (♂) the larger Biserrate. (After* THEOBALD)

The Ungues.—The ungues vary in the male and female and in the different legs. They may be simple, uniserrated or biserrated (rarely triserrated). (Fig. 45.) They are of specific value (THEOBALD).

THE ABDOMEN

The abdomen consists of nine segments. To the ninth segment are attached the genitalia.

The genitalia are variously shaped lobed appendages. In the male they are provided at their free end with claspers. The claspers in the male are of specific value (Fig. 46).



Fig 46 Male Genitalia

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2. Nuttall and Shipley. *Journal of Hygiene* Vol I, No. 1. Vol. I, No. 4 Vol. III, No 2 *The Anatomy of the Larva, Pupa, and Imago* Well illustrated.

3. *Liverpool School of Tropical Medicine* Memoir IV, Part II. 'Filariasis,' including a minute study of the proboscis of the mosquito. Many illustrations (Price 10s 6d)

Chapter XV

CLASSIFICATION AND IDENTIFICATION OF THE CULICIDAE

SCALES

THEOBALD has attached to scale structure the greatest importance from the point of view of generic and specific classification. Hence it is necessary to consider somewhat in detail these structures. THEOBALD gives the following:—

Head Scales.—Three forms of scale occur (Fig. 47).

1. Narrow curved scales.
2. Upright forked scales.
3. Flat scales overlying one another like the tiles of a roof.

No. 1, 2, and 3 scales found, *e.g.*, *Culex.*
No. 2 and 3 scales only, found = *Stegomyia.*
No. 3 scales only, found = *Megarhinus.*
Toxorhynchites.

Thoracic Scales.—THEOBALD describes five forms (Fig. 47).

1. *Narrow Hair-like Curved Scales.*—They often form a dense feltwork over the mesothorax.

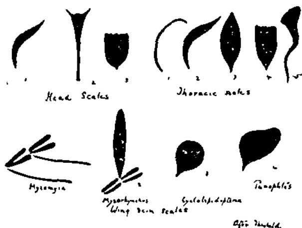
2. *Narrow Curved Scales.*—They may occur all over the mesothorax and scutellum, or at the sides of the scutum and in front of the scutellum.

3. *Spindle-Shaped Scales.*—These lie scattered about, and never form a complete covering.

4. *Flat Scales* like those on the head. They cover the scutellum in *Stegomyia*, whereas in *Culex* the scutellar scales are of the narrow curved type.

5. *Long Twisted Scales.*—Characteristic of *Mucidus*, a genus of mouldy-looking mosquitoes.

Abdominal Scales.—The scales covering the abdomen in all *Culices*, except the *Anophelina*, are overlapping flat scales. In the *Anophelina* they are not found, except to some extent in *Myzorhynchus* and *Nyssorhynchus*. In the genus *Aldrichia*, however, of *Anophelina*, the abdomen is covered with flat scales as in *Culex*.



[*Fig.* 47. *Varieties of Scales* (*after* THEOBALD).
Panoplites = *Mansonia*

The *Culicidae* are divided into the following sub-families, based mainly upon the length of the palpi in male and female.

1. Palpi long in both sexes, as long as the proboscis in the female - - - *Anophelina*

2. Palpi long in both sexes, shorter than the proboscis in the female - - *Megarhinina*

3. Palpi short in female, long in male— *Culicina*

4. *Palpi short in female, long in male.* Post-scutellum with hairs (chaetae) and scales, *Joblotina* [= *Trichoprosopina*

5. Seven (not six) longitudinal veins with scales - - - - - - *Heptaphlebomyina*

6. Palpi very short in female and male— *Aedeomyina*

7. Proboscis short, not formed for piercing - - - - - - *Corethrina*

Wing Scales.—Scales clothe the veins, except the cross veins. Flat scales are arranged in a double row along each vein.

Many, or in some species all, of the veins have also lateral scales.

The lateral scales are very variable in shape, *e.g.*,

1. In *Mansonia* they are broad asymmetrical flat scales.

2. In *Aedomyia* the scales are similar.

3. In *Mucidus* the wing scales are quite chracteristic, being pyriform or inflated and half-dark, half-white.

4. In *Megarhinus* the scales may be azure green or blue.

The Wing Fringes consist of—

1. Long narrow-pointed scales attached to the edge of the wing by a narrow stalk.

2. Smaller scales similar in shape.

3. Border scales. Small flat scales.

Leg Scales.—The legs are covered with flat scales in nearly all *Culices.*

1. In *Sabethes* the scales are hair-like and occur in tufts.

2. In *Mucidus*, *Psorophora*, the scales are elongated and project from the legs.

The sub-family *Anophelina* contains, as we shall see, seven genera, the *Culicina* fifteen, and the *Aedeomyina* twenty. When we consider, further, the large number of species in some of these genera, *e.g.*, *Culex*, it is impossible to attempt here to describe each mosquito, however briefly. Considering the great importance, however, of the *Anophelina*, we shall attempt to give the characteristic specific points for each of the species, as an aid to a detailed examination by means of THEOBALD'S monograph. With regard to the other sub-families we shall attempt only to give characteristics of each genus.

SUB-FAMILY ANOPHELINA (*vide* next Chapter)

SUB-FAMILY MEGARHININA

Genus 1. *Megarhinus*.—First sub marginal cell much smaller than second posterior cell Palpi

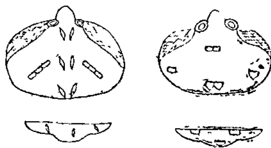


Fig. 48. Head and Scutellar Scales of Aedes (left) Megarhinus (right)

five-jointed in ♀ (*M. purpureus* only four). Readily recognized: (i) by their large size, often called 'elephant mosquitoes'; (ii) by their brilliant metallic colours; (iii) by a caudal tuft of hairs on each side of the abdomen; (iv) by the long and curved proboscis; (v) head is clothed with flat scales only (Fig. 48).

They may be found resting on the trunk of trees in the forest, also in houses in the bush. Species, about six.

Genus 2. *Toxorhynchites.*—Palpi much shorter than proboscis in ♀, three-jointed. Supernumerary cross-vein nearer the apex of the wing than the mid cross-vein. Species, four.

SUB-FAMILY CULICINA

First sub-marginal cell equal to or longer than the second posterior cell.

Genus 1. *Janthinosoma.*—*Hind* legs densely scaled, giving a characteristic appearance. Species, five.

Genus 2. *Psorophora.*—Characterized by (i) great length of ♂ palpi, five-jointed; (ii) densely long scaled legs; (iii) posterior cross-vein a little nearer the base than the mid; (iv) proboscis curved in ♀. Species, four.

Genus 3. *Mucidus.*—Easily recognized by their curious mouldy appearance. Posterior cross-vein nearer apex of wing than mid. Wing scales large, pyriform, parti-coloured. Head and thoracic scales long and twisted, expanded at the apex. Legs densely scaled with projecting scales. Species, five.

Genus 4. *Desvoidea = Armigeres.*—Head, flat scales, a few upright-forked. Differs from *Stegomyia*, (i) is longer, with unbanded tarsi and abdomen. ♂ palpi, untufted. ♀ palpi, very pointed and provided with bristles only. Species, two.

Genus 5. *Stegomyia.* — Head completely clothed with broad flat scales (Fig. 49) and a few upright forked. Palpi four-jointed in the ♀, five-jointed in the ♂. *Scutellar scales flat*, mostly black and white mosquitoes with banded legs and abdomen. Species, eighteen.

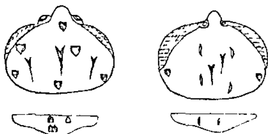


Fig. 49. Head and Scutellar Scales of Stegomyia (left) and Culex (right)

S. *Fasciata.* Transmits yellow fever

1. Tarsi *basally* banded white.
2. Proboscis unbanded.
3. Thorax. A pure white broad curved band on each side, and two median pale parallel lines.
4. Ungues of ♀ toothed.

Genus 6. *Theobaldia.* Palpi in ♂ clubbed as in the *Anophelina.* Palpi in ♀ five jointed, apical joint mammilliform, wings in both sexes densely

scaled, collected into spots, thus forming a *spotted wing* group of mosquitoes. Species, five.

Genus 7. *Lutzia.*—Resembles *Theobaldia.*

Palpi in ♀ three-jointed, apical joint not mammilliform.

Palpi in ♂ not clubbed, three-jointed.

Wings spotted by scales similar to those of *Taeniorhynchus.* Species, one.

Genus 8. *Culex.*—Head scales: *narrow curved and upright forked*; laterally flat scales (Fig 49). Palpi in ♀, three-jointed. Third palpal joint usually as long or longer than the other two.

Wing: first sub-marginal cell longer and narrower than the second posterior. Posterior cross-vein nearer the base than the mid wing-veins. Scales small, lateral ones linear.

Scutellum: *narrow curved or spindle scales* (Fig. 49).

C. mimeticus has spotted wings. Species, very numerous.

Genus 9. *Gilesia.*—Related to *Culex* and *Stegomyia.*

(i) Scutellum *with small flat scales,* some spindle scales.

(ii) Head: broad, flat, spindle scales.

(iii) Basal joint of antennae, hairy and scaly.

(iv) Claws short and thick with a blunt tooth.

(v) Wing scales like those of *Taeniorhynchus.* Species, one.

Genus 10. *Lasioconops.*—

(i) Head scales as in *Culex.* Basal joint of antennae, a few scales.

(ii) Abdomen: *large projecting flat scales with deeply dentate apices*, giving these mosquitoes a ragged appearance. Species, one.

Genus 11. *Melanoconion*.—Distinguished from *Culex* by the dense broad scales on the costa and apex, and by the black spine-like scales along the upper border. Small dark mosquitoes. Species, six.

Genus 12. *Grabhamia*.—Allied to *Culex* and *Taeniorhynchus*. Palpi in ♀ four-jointed. Apical joint minute. Penultimate long and thick. Wing scales not so long or dense as in *Taeniorhynchus*. Scales mottled. Wings short and stumpy. Legs mottled and spotted. Species, ten.

Genus 13. *Acartomyia*. Allied to *Culex* and *Grabhamia*. Distinguished from *Grabhamia* by having *flat irregularly disposed scales* all over the head, from *Culex* in the ♂ palpi. Two terminal segments and the apex of the antipenultimate swollen. Terminal segment club shaped. Ragged appearance of head, well marked. Species, one.

Genus 14. *Taeniorhynchus*. Palpi five-jointed in ♂, the fifth segment minute. Characterized by the wing scales. They are *thick elongated scales ending with a broad sloping concavity or blunt point*, median linear scales often absent, proboscis usually banded. Species, about sixteen.

Genus 15. *Mansonia* = *Panoplites*. Palpi four-jointed in ♂, more than one-third the length of the proboscis. Characterized by *wings densely scaled along the veins with broad asymmetrical flat scales*. *No median scales*. The genus resembles *Aedeomyia*, but the palpi in the ♂ are long in members of this genus, short in the *Aedomyina*. Species, eight.

Sub-Family Joblotina

Genus 1. *Joblotina*.—Metanotum (= Post-scutellum) *with a tuft of chaetae and with flat scales.* Clypeus and base of antennae bristly. Second long vein carried nearly to the base of the wing. *Second posterior fork cell (anal cell) very* large. Mid cross-vein nearer the apex than the anterior (supernumerary). Wings densely scaled; scales shorter than in *Taeniorhynchus*. Species, one.

Sub-Family Heptaphlebomyina

Genus 1. *Heptaphlebomyia*.—Like *Culex*, but has a distinct seventh vein. Species, one.

Sub-Family Aedeomyina

Genus 1. *Deinocerites* = [*Brachiomyia*].—Characterized by the ♀ antennae. Much longer than the proboscis. *Second segment as long as the three terminal segments.* Antennae scaled. Antennae in ♂ pilose and longer than the whole body. Species, two.

Genus 2. *Finlaya*.—Three ventral abdominal scale tufts. Scutellum, four median bristles. Wing scales, large and broad, pyriform. Species, two.

Genus 3. *Aedes*.—Head, narrow curved scales form a *broad median line only*. Other scales flat. Scutellum, *narrow curved scales*, six bristles. Palpi in ♀, four segments, apical segment minute, mammilliform. Traces of a fifth segment. Species, two (Fig. 48).

Genus 4. *Howardina*.—Resembles *Aedes*, but scutellum has only four bristles. Palpi, four

segments, apical, one minute, not mammilliform. Species, two.

Genus 5. *Acdimorphus.*—Head, mostly flat scales, narrow curved behind. *Scutellum, flat scales*, eight (?) bristles. Has no flat thoracic scales as *Uranotaenia*. Species, one.

Genus 6. *Skusea.*—Head, *flat scales only*. Anterior and posterior forked cells densely scaled. Palpi in ♀, three segments. Scutellum, six bristles and narrow curved scales. Species, three.

Genus 7. *Verrallina.*—Head as in *Skusea*. Palpi, two segments only (trace of a third), apical segment large. Scutellum, four bristles and narrow curved scales. Species, three.

Genus 8. *Ficalbia.* Intermediate between last two and next genus. Head scales, *no narrow curved*, almost entirely flat. Scutellum, *flat scales* as in *Uranotaenia*, but *thoracic scales narrow curved*. Palpi, two segments. Species, two.

Genus 9. *Uranotaenia.*—Head, *flat scales*, upright forked may or may not be present. Scutellum *flat scales*. Thorax, *narrow curved and flat scales*. Wings, small forked cells. Metallic scales at the base of the wings. Related to *Aedes*, but more brilliant (metallic) and stouter mosquitoes. Species, fourteen.

Genus 10. *Myomyia.*—Resembles *Uranotaenia*. Has no flat scutellar or thoracic scales. Forked cells larger than *Uranotaenia*. No metallic scales at the base of the wings. Species, two.

Genus 11. *Aedeomyia.* Allied to *Aedes*. Distinguished by (i) head scales upright, fan-shaped; clypeus scaly; (ii) thorax, broad, flat spindle scales; (iii) scutellum, broad flat scales; (iv) legs,

densely scaled; (v) wings, densely scaled as in *Mansonia*, also with long lateral scales. Species, three.

Genus 12. *Haemagogus.*—Related to *Aedes*, but palpi five segments. Head covered with flat scales. Brilliant metallic (blue) mosquitoes. Species, two.

Genus 13. *Wyeomyia.*—*Chaetae on the post-scutellum.* Head, flat scales. Thorax, spindle and flat scales. Scutellum, flat scales. Palpi short. *Proboscis not as long as whole body.* Species, two.

Genus 14. *Phoniomyia.*—Resembles *Wyeomyia*, but distinguished by (i) wing scales broad, lateral scales as in *Taeniorhynchus*; (ii) proboscis longer than the whole body. Species, two.

Genus 15. *Dendromyia.*—Resembles *Wyeomyia*, distinguished by (i) scutellar scales small, flat, rounded apically; (ii) wings more densely scaled than in *Phoniomyia*, scales *Taeniorhynchus*-like; (iii) proboscis moderately long. Species, five.

Genus 16. *Runchomyia.*—Allied to *Dendromyia*. Characterized by (i) frons projecting as a blunt spine; (ii) proboscis as long as the body in ♀; (iii) ventral apical tuft of bristles; (iv) wings covered with rather broad scales. Species, one.

Genus 17. *Sabethes.*—Distinguished from *Wyeomyia* by the *asymmetrical wing scales.* One or more *legs with dense paddle-like structures* in both sexes. Mid cross-vein nearer the apex than the anterior. Posterior nearer the apex than the mid in the ♂. Third long vein carried through into the basal cell. Brilliant metallic mosquitoes. Species, four.

Genus 18. *Sabethoides.*—Closely resembles *Sabethes.* Distinguished (i) by much smaller palpi; (ii) unpaddled legs. Species, one.

Genus 19. *Goeldia.*—Post-scutellum with *chaetae and scales.* Wing scales as in *Runchomyia,* dense, elongated. Wing venation as in *Culex.* Proboscis short and thick; not as long as body. Palpi in ♂ one-third length of the proboscis. In ♀ quite short. Species, one.

Genus 20. *Limatus.*- Characterized by the proboscis bent in the middle; densely scaled at the bend. Species, one.

SUB-FAMILY CORETHRINA

Genus 1. *Corethra.*—First tarsal segment longer than the second tarsal.

Genus 2. *Mochlonyx.*—First tarsal segment shorter than the first tarsal.

LITERATURE

A Monograph of the Culicidae Vols I-III. F. V. THEOBALD. *Brit Mus Nat Hist* The data in this chapter have been taken almost entirely from THEOBALD's work.

Chapter XVI

THE CLASSIFICATION AND IDENTIFICATION OF THE ANOPHELINA

It is by no means an easy matter to fix definitely the *species* of an *Anopheles*, and yet the identification of species is essential in connexion with malarial studies. It does not suffice merely to ascertain that *Anopheles* are present in any given locality, but it must be clearly made out what the *species* are. It is, indeed, only by accurately observing the relation of *Anopheles* to malaria that we can hope for the explanation of many of the difficulties surrounding the anomalous distribution of endemic malaria. In a later chapter the extreme importance of the species of *Anopheles* in this connexion will be evident. In the description of the habits of *Anopheles*, their breeding places, occurrence apart from man, etc., it is no longer sufficient to ascribe these to the whole genus, but they must be ascribed to the actual species involved. The study of the *Anopheles* from the point of view of classification and identification is, therefore, of importance.

Classification of Anophelina

Theobald has sub-divided the genus *Anopheles* into ten new genera. These genera comprise

over seventy-eight different species. The assigning of an *Anopheles* (old sense) to its proper genus simplifies, therefore, very much the ultimate determination of the particular species. THEOBALD's classification is the following:—

Thorax and abdomen with hair-like curved scales	Prothoracic lobes simple, no flat head scales	Wing scales lanceolate -	*Anopheles*
		Wing scales mostly long and narrow -	*Myzomyia*
		Wing scales partly large and inflated	*Cyclolepidopteron*
	Prothoracic lobes mammillated, median flat scales	Wing scales lanceolate -	*Stethomyia*
Thorax with narrow curved scales, abdomen hairy	Wing scales small lanceolate or narrowed	-	*Pyretophorus*
Thorax with hair-like curved scales, some narrow curved ones in front, abdomen with apical lateral scale tufts and scaly venter, no ventral tuft			*Arribalzagia*
Thorax with hair-like curved scales, abdominal scales on venter only, with a distinct ventral apical tuft, no lateral tufts			*Myzorhynchus*

Thorax and abdomen with true scales	Abdominal scales as lateral tufts and dorsal patches of small flat scales; thoracic narrow curved or spindle-shaped - - - -	*Nyssorhynchus*
	Abdomen nearly completely scaled with irregular scales and with lateral tufts - -	*Cellia*
	Abdomen completely scaled with large flat scales, as in *Culex* - - - - -	*Aldrichia*

These features are shewn in the accompanying diagram (Fig. 50). The thoracic scales in *Cyclolepidopteron* are not sufficiently hair-like (THEOBALD).

Place the mounted or unmounted mosquito under a low power of the microscope, and determine carefully the characters of the hairs or scales on the head, thorax and abdomen. By this means the *Anophelete* is assigned to its proper genus.

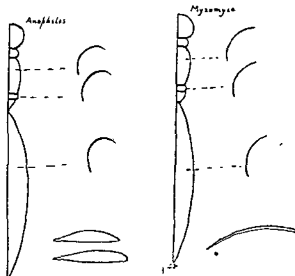


Fig. 50 *Thoracic, Scutellar, and Abdominal Scales of the Anophelina* (after THEOBALD)

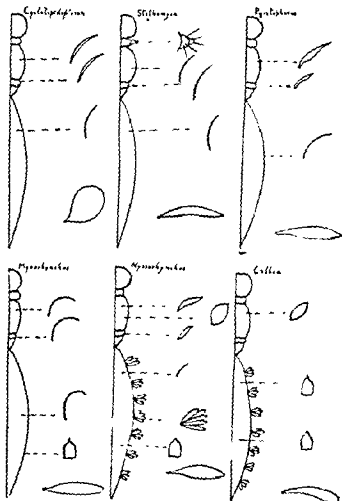


Fig. 50.—Contd. Thoracic, Scutellar and Abdominal Scales of the Anophelina (after THEOBALD)

The Differentiation of Species

Many features are of value in determining the species.

1. *The Wings*:—

(*a*) They may shew areas of dark scales on the costa, auxiliary, and first longitudinal veins, producing the main spots of the wing.

(*b*) Small areas of scales on the third to sixth long veins; less dark and distinct than the large costal spots.

(*c*) Pale areas on the wing fringe.

(*a*) The markings on the wing are fairly constant in each species, but variations occur, so that the spot may be longer or shorter, giving the wing a darker or lighter aspect in the same species. Thus in *N. Stephensi* the following variations in the second costal spot may be encountered (Fig. 51). Especially does this variation occur in the wing of males. The costal spots may also be confluent. They may depart from their typical shape, as is frequently seen in the T spot of *M. Rossii*. Certain general types of wing can be recognized:—

(i) Wings almost entirely covered with black scales, as in the genus *Myzorhynchus*.

(ii) Wings only slightly spotted on the costa or wing field, *e.g.*, in the genus *Anopheles*.

(iii) Wings with well-marked discrete spots, (*a*) brownish wings as in *Myomyia*; (*b*) black wings as in *Nyssorhynchus*.

(*b*) The smaller spots on the wing field along the course of the veins are also useful for determining species. Thus *M. leucophyrus* has six spots on the sixth long vein, while *M. elegans* has only four. The extent to which the third longitudinal vein is scaled is also of specific importance (Fig. 52).

(*c*) The wing fringe has at the points, where the long veins cut the margin, a variable number of light areas. Thus *A. punctipennis* has only one pale area, while *A. pseudopunctipennis* has many. Another example of this means of distinguishing species is given in the figure (Fig. 51).

2. *Leg Markings*:—

(*a*) Uniformly coloured as in the second division of *Myzomyia*.

(*b*) Speckled or banded, chiefly in the genera *Nyssorhynchus* and *Cellia*. The banding of the legs is of great importance in distinguishing the species (Fig. 53); thus (1) banded tarsi, *e.g.*, *N. Maculata*; (2) tarsi pure white, *e.g.*, *N. fuliginosus*, *N. Jamesii*.

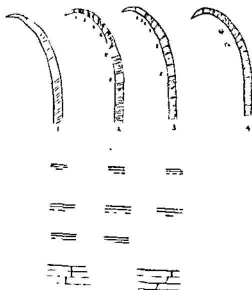


Fig. 51 *Wing fringes of* (1) *M. Rhodesiensis*, (2) *M. Funestus*, (3) *M. Listoni*, (4) *M. Culicifacies*
Variations in Wing Spots of M. Rossi, N. Stephensi, and P. Costalis
Variations of Cross-veins of M. Rossi

The Differentiation of Species

Many features are of value in determining the species.

1. *The Wings*:—

(*a*) They may shew areas of dark scales on the costa, auxiliary, and first longitudinal veins, producing the main spots of the wing.

(*b*) Small areas of scales on the third to sixth long veins; less dark and distinct than the large costal spots.

(*c*) Pale areas on the wing fringe.

(*a*) The markings on the wing are fairly constant in each species, but variations occur, so that the spot may be longer or shorter, giving the wing a darker or lighter aspect in the same species Thus in *N. Stephensi* the following variations in the second costal spot may be encountered (Fig. 51). Especially does this variation occur in the wing of males. The costal spots may also be confluent. They may depart from their typical shape, as is frequently seen in the T spot of *M. Rossii*. Certain general types of wing can be recognized:—

- (i) Wings almost entirely covered with black scales, as in the genus *Myzorhynchus*.
- (ii) Wings only slightly spotted on the costa or wing field, *e.g.*, in the genus *Anopheles*.
- (iii) Wings with well-marked discrete spots, (*a*) brownish wings as in *Myomyia*; (*b*) black wings as in *Nyssorhynchus*.

(*b*) The smaller spots on the wing field along the course of the veins are also useful for determining species. Thus *M. leucophyrus* has six spots on the sixth long vein, while *M. elegans* has only four. The extent to which the third longitudinal vein is scaled is also of specific importance (Fig. 52).

(*c*) The wing fringe has at the points, where the long veins cut the margin, a variable number of light areas. Thus *A. punctipennis* has only one pale area, while *A. pseudo-punctipennis* has many. Another example of this means of distinguishing species is given in the figure (Fig. 51).

2. *Leg Markings*:—

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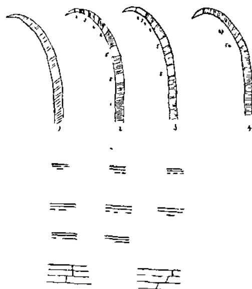


Fig. 51 *Wing fringes of* (1) *M Rhodesiensis*, (2) *M Funestus*, (3) *M. Listoni*, (4) *M. Culicifacies*
Variations in Wing Spots of M. Rossii, N Stephensi, and P Costalis
Variations of Cross-veins of M. Rossii

3. *Palpi*:—Similarly the bands or collection of white scales on the palpi is a convenient means of separating the members of a particular genus (Figs. 52, 53). It should not be forgotten that in these two characteristics there is a certain amount of variation, possibly seasonal, and a slight difference in the bands on the palpi and legs is not sufficient in itself to constitute a difference in species.

Other characteristics that are useful for the determination of species are the male genitalia, and the character of the ungues in the male, whether having one or more teeth. The position of the cross-veins has also been used, but this is so variable in the same species that it has little value.

Characters of the Larvae and Ova.—In the *Anophelina*, as in the rest of the *Culicidae*, this is a most important means of differentiation. Mosquitoes that otherwise are almost indistinguishable are readily separated by their larvae being different.

One precaution must be taken. It must be quite certain that it is the larva of the mosquito in question that is being examined. The easiest way to make sure of this is to carefully examine the larva first, and then to hatch out the mosquito and then examine it. The examination of the larvae is considered later.

Genus 1. *Anopheles.*—Wings unspotted or slightly spotted. Larva: clypeal hairs branched. Ova, type 1. Mosquitoes not particularly domestic in habits. Mostly belong to temperate climes or hill districts.

A. Bifurcatus transmits malaria (in Italy). Zygotes have been found in *A. maculipennis* in Spain, but experiments with this species in England have failed, though successful on the continent. They may be divided up in the following way:—

Costa *Uniform*, *Wings spotted*.

1. *A. Maculipennis.*—Wing with four spots; apex of first tarsal joint spotted. Europe.

2. *A. Crucians.*—White spots on brown veins. Three black spots on sixth vein. Costa uniformly dark; tarsi unbanded. North America.

Costa Spotted

3. *A. Punctipennis.*—Two yellow spots, one at the apex, the second on the apical third. One fringe spot. North America.

4. *A. Pseudo-Punctipennis.*—Wings as *in previous* species; but wing fringe with several yellow spots. North America.

5. *A. Gigas.*—Costa, two large black spots. Length, five to six mm A large hill species. India

6. *A. Lindesayii.*—Costa black, apical white spot. Femora have a characteristic broad median white band. A hill species. India.

Wings Unspotted

7. *A. Bifurcatus.*—Thorax. Golden hairs arranged so as to leave two broad bare lines on the front. *Abdominal hairs golden.* Europe.

A. Walkeri is regarded by THEOBALD as identical with this.

8. *A. Algeriensis.*—Abdominal hairs dull brown. First forked cell shorter than in *A. Bifurcatus.*

9. *A. Nigripes.*—A black mosquito. No bands on tarsus. Europe. America.

10. *A. Immaculatus.*—Ash-grey in colour. Slight apical bandings to tarsi. Palpi and proboscis lighter at apex. Ennur, Madras. A single species only found by us.

11. *A. Aitkenii.*—Uniformly dark. No markings on palpi or legs. Bombay Presidency.

12. *A. Stigmaticus.*—Light brown. Australia.

13. *A. Philippinensis.*

Genus *Myzomyia.*—To this genus belong those species which are associated in the tropics with the most severe endemic malaria, *e.g.*, *M. Funesta* in Africa and *M. Listoni* and *M. culicifacies* in India. The group includes, however, several species, one at least of which has, as far as our knowledge extends, no power of transmitting malaria in nature, viz., *M. Rossii.*

The malaria transmitters form a natural group: they are small, dark mosquitoes, with unbanded legs, and they breed in fresh natural waters, *e.g.*, streams, river beds, etc.; whereas we also have in the group domestic mosquitoes, *i.e.*, those that breed in foul pools about houses. *M. Rossii* is the type of this class.

Whether in this genus any others than the three mentioned above convey malaria there are at present no facts to shew, and the larval characters of only the Indian species are at present known.

The type species is *M. Funesta*, which is a typical spring and fresh-water breeder. It is noteworthy that *M. Funesta* is associated with a higher malarial endemicity than *P. Costalis*, which is a typical domestic mosquito breeding in foul pools.

GROUP I

Small dark mosquitoes breeding in natural waters.

1. *M. Funesta.*—Costa: six white spots. Basal spots with pale interruption. Wing fringe: pale spots at ends of all the veins, except sixth. Palpi: three bands, the basal one further from the middle one than the apical. A variable species: third long vein may be dark. Resembles *Listoni* and *Rhodesiensis* (Fig. 51)

2. *M. Listoni.*—*Third long vein light.* Wing fringe, four or more light spots (Fig. 51). Palpi, two broad apical bands further apart than in *Funestus*, one narrow basal. Basal portion of costa uniformly black (characteristic). Attitude, *Anopheles*-like. Associated with high endemic index in the Duars, Bengal. Larval characters: antennae with simple hair. Clypeal hairs simple. Palmate hairs on thorax and on all abdominal segments.

3. *M. Aconita.*—*Aconita* = unspeckled, because at the commencement of the third long vein the usual dark spot is absent. Palpi, four bands. Costa, four spots, light interruption in basal spot. Fringe, several pale areas. Anterior forked cell much longer and narrower than posterior. Differs from *Listoni* in palpi. Sumatra, Java.

4. *M. Culicifacies.*—*Third longitudinal vein dark.* Wing fringe, three spots at most. Palpi, three equal bands, two at the joints, one at the apex. *Attitude, Culex-like.* Associated with high endemic index of malaria in the Punjab and Madras. Larval characters as in *Listoni.* Ova: floats do not touch margin of upper surface (Type 1) (Fig. 51).

5. *M. Leptomeres.*—Base of first long vein white. Anterior forked cell much longer and narrower than posterior. Costa, two spots, thus differing from *Hebes.* Fringe, pale areas at all the veins.

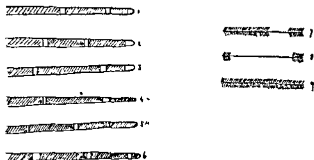


Fig. 52
Palpi of M. Funesta (1), *M. Listoni* (2), *M. Culicifacies* (3), *M. Rhodesiensis* (4), *M. Hispaniola* (5), *M. Turkhudi* (6), *Third Long Veins of M. Funesta* (7), *M. Listoni* (8), *M. Culicifacies* (9)

6. *M. Hebes.*—*Hebes* = inconspicuous, a small species resembling *Rhodesiensis.* Wing costa, four spots; wing fringe, seven light areas. Vein six, one long spot. Palpi, first and second segments covered with white scales. End of third segment is dark, fourth segment quite white. Distinguished from *Rhodesiensis* by palpi and wing fringe. East Africa.

Group II

Larger species than the above. Generally lighter. Wings not so covered with dark scales.

Habits: Domestic species, breeding in foul puddles, etc., near houses.

Larval characters (*M. Rossii*) : Antenna without branched lateral hairs. Clypeal hairs simple. Palmate hairs second to seventh segments, and often rudimentary hairs on thorax and first and second abdominal segments. Ova (type 2), *i.e.*, floats, touching margin of anterior surface.

7. *M. Albirostris*—Characterized by the banded proboscis; pale scaled to about half its length. *N. deceptor* has also a banded proboscis. Malaysia. Length, two to five mm.

8. *M. Longipalpis*—Palpi long, thin; three narrow white rings; wing costa, black, four almost equal yellow spots; wings mostly brown scaled; hind legs only, banded; narrow apical and basal yellow bands. British Central Africa. Length, three mm.

9. *M. Ludlowii.*—Palpi, apex broad white band; a second small one close to it; a third basal band. Wing costa, four large spots, one or two small basal. Legs, femora, tibiae and metatarsi, especially in hind legs: *spotted* with yellow. Tarsi, broad apical and basal pale banding, especially in hind legs. Philippine Isles. Length, four to five mm.

10. *M. Rossii.*—Probably = *A. Vagus* (Dönitz). Sumatra. Palpi, somewhat like those of *M. Ludlowii*, but easily distinguished, the apical white band is broader; the second band is much nearer the base than in *M. Ludlowii*, so that the black area between is longer. Wings, four spots, and some basal spots. The second large spot has the characteristic *T. shape*, but is very variable. Tarsi, slight pale apical and basal bands to some of tarsi. India, Malaysia.

11. M. *Lützii.*—Characterized by the linear ornamentation on the thorax, and marked bands (five in number) on the fore and mid metatarsi. Wings, three distinct pale spots; two smaller ones, 3 to 3·5 mm. Rio de Janeiro.

12. *M. Elegans.*—Possibly = *N. Leucophyrus* (DÖNITZ). Leukos = white, sphyron = ankle-joint. Palpi, *four white bands.* Wing costa, four large black-scaled areas, three small. Wing fringe, six pale interruptions. Legs speckled with white scales. Femora and tibiae speckled in hind legs. *Characterized by a large tibio-metatarsal* band on the hind legs. Resembles *N. Stephensi*; differs in the palpi; has four, not three, spots on the sixth longitudinal vein. Differs from *N. Leucophyrus* in having four, not six, spots on the sixth vein. Possibly belongs to *Nyssorhyncus* genus. India.

13. *M. tesselatum.*—Costa, four large, four small spots. Fore tarsi apically and basally banded. Mid and hind tarsi apically only. Thorax, two dark spots in front and a dark area near the scutellum. Malay.

14. *M. punctulatus.*—Costa, four large spots and numerous dark and white spots. Malay; closely resembles the former.

15. *M. Leucophyrus.—Related to the two* former. Distinguished by prominent tibio-metatarsal band and by the prominent median dark spot on costa. Sumatra.

16. *M. Impunctus.* Costa, four small dark spots. Fringe spotted. Sixth vein, three spots. Relationship doubtful; not fully described. Egypt.

Group III

Medium size dark mosquitoes. Apex of palpi black.

17. *M. Turkhudi.*—Palpi, *apices black*, the band not so broad as in Hispaniola; third long vein mostly dark, but varies; pale interruption in basal costal spot. India. Larvae resemble *Culex*. Ova, very peculiar, type 3 (*vide* p. 222).

18. *M. Hispaniola* (Theo.) Spain. Third longitudinal vein, *mostly pale yellow*, *except at* the base and apex. Wing fringe with spots, except where lower branch of fifth and sixth join the costa. Basal portion of costa uniformly black.

19. *M. Rhodesiensis* (Theo.) Rhodesia.—Third longitudinal vein dark. Palps with only two conspicuous bands. The palpi are much longer and thinner than in *M. funestus*. The veins are all dusky scaled. Base of the costa black. In *M. funestus* there is a white interruption. Wings, costa three small white spots and a *yellow apical spot*. *Fringe unspotted*, except an apical spot (Fig. 51)

Genus *Cyclolepptcron.*—Wings with numerous large inflated scales; collected in patches or irregularly disposed

Larval Characters (Theobald).—Antenna without lateral branched hair. Clypeal hairs simple. Palmate hairs, six pairs Lanceolate.

1. *C Grabhamii* - Palpi unbanded. Jamaica.
2. *C. Mediopunctatus.*—Palpi banded, black and gold. Brazil.

Genus *Stethomyia*(στηθος = breast).—Head with a median patch of flat scales. Palpi very thin.

1. *S. nimba*. British Guiana, S. America.

1. *A. Maculipes.*—Hind and mid legs much banded and speckled. Almost certainly transmits malaria (LUTZ).

Genus *Myzorhynchus.*—μύζω to suck, ῥύγχος, proboscis.

Palpi densely scaled in the ♀, also the proboscis. These are 'wild' mosquitoes found in situations remote from the dwellings of man. They breed in swamps and large bodies of water, especially those containing weeds. They do not usually frequent houses. *M. Sinensis* is, however, attracted by light. They feed readily on human blood when occasion offers.

Larval Characters. — They occur singly throughout large masses of water. They have a peculiar stiff and stick-like appearance, but they also exhibit curious attitudes, sharp bends, as they lie on the water. Clypeal hairs distinctive, the outer pair forming a dense cockade-like tuft.

The antenna is characterized by possessing a large branched hair arising from a papilla on its side.

The palmate hairs are borne upon the third to seventh abdominal segments. The leaflets are highly characteristic, being unlike those of the other genera (so far as described), lanceolate and serrated.

The Characters of the Ovum.—In *M. barbirostris* and *M. Sinensis*, the ova belong to type 1, *i.e.*, the lateral floats not touching the marginal rim.

In *M. barbirostris* and *M. Sinensis*, there is a characteristic appearance of a well-marked polygonal pattern upon the under surface.

(A) Palpi *unbanded.*—Last hind tarsus brown:

1. *M. barbirostris*, one fringe spot. India, Malaysia.

2. *M. Bancroftii*, several fringe spots. Australia.
3. *M. umbrosus*, no fringe spot, only one costal spot. Malaysia.

Last hind tarsus white:

4. *M. albotaeniatus*, other hind tarsi much banded. Malay.

Last two hind tarsi white:

5. *M. Constani*. Madagascar.

(B) Palpi *banded*.—Last hind tarsus brown:

6. *M. Sinensis*, wing fringe, one pale spot. China

Palpi banded, last hind tarsus brown, wing fringe unspotted. Apex of palpi white:

7. *M. Vanus*, costa two yellow spots, wings distinctly spotted. India, Malay, Philippines, etc.
8. *M. Pseudopictus*, wings without prominent spots. Europe.
9. *M. minutus*, wings, two white costal spots. Punjab

Apex of palpi black:

10. *M. nigerrimus*. India.

(C) Palpi banded, last hind tarsi white

11. *M. mauritianus*, two hind tarsi white.
12. *M. Paludis*, three hind tarsi white. Africa.

Note.—DÖNITZ *Plumiger* and *Tenebrosus* are either *Barbirostris* or *Sinensis* according to THEOBALD Add *M Pseudobarbirostris*, from the Philippines

Genus *Nyssorhynchus*.—νύσσω, to puncture, 'bite,' ῥύγχος, proboscis.

Mosquitoes mostly with legs spotted and banded, or one or more tarsal segments pure white. They are both domestic and wild mosquitoes. They breed chiefly in pools with algae,

and in lakes. N. *Stephensi* will, however, breed in pots and tins. Larva: antennal hair simple. Ova, type 2.

Legs *unspotted*. Larva with outer pair of clypeal hairs markedly branched. Leaflets of palmate hairs with long filament.

1. *N. Fuliginosus.* — Probably = *Leucopus*, DÖNITZ. Costa, four large and one or more small pale spots. Femora, pale band near the apex. Hind tarsi, three and one-fifth pure white. Palpi, two narrow white bands, apex white (Fig. 53). India.

2. *N. Karwari.*—Legs not speckled, one and one-fourth hind tarsal joints white. In fore and mid legs, tarsal joints, except fourth and fifth, have apical white band. In hind legs, tibia, first and second tarsus, have apical bands, third and fourth have both apical and basal bands; the fifth is white. Palps, four white bands, two terminal broad and equal, two basal narrow, apex white. India.

Legs markedly *spotted*. Larva with simple or slightly branched frontal hairs. *Filaments* of *palmate* leaflets *very short*.

3. *N. Stephensi.*—Syn = *A. Metaboles*, THEOBALD. Tarsus without any segment of hind leg white. Legs brown, speckled with white; joints of fore and hind tarsi with apical spots. Wing costa, four broad prominent black spots and two smaller basal ones. The third largest spot has three typical spots beneath it on the first long vein. Fringe dark, with pale areas. Palpi, two broad apical white bands, one narrow basal; white scales between the last two bands. India.

4. *N. Maculatus.*—Resembles *N. Stephensi*, but is easily distinguished by tarsi. Wings, costa four large and two small basal spots. Under the third largest spot are three black spots on the first long vein. Legs, with femora, tibiae, and metatarsi with broken creamy bands and spots. Fore and mid tarsi with narrow yellow bands. Hind tarsi with broad white ones. *Last segment pure white.* Palpi, four bands, two unequal white apical bands, then a small white one, and a second towards the base.

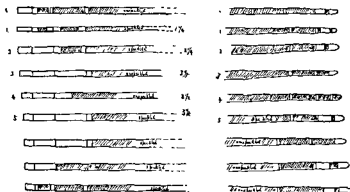


Fig 53

Hind Tarsi and Palpi of N Karwari (6), *N Maculata* (1), *N. Theobaldi* (2), *N Fuliginosus* (3), *N Fuliginosus (Nagpurensis)* (4) *N Maculipalpis* (5), *N Jamesii* (7), *N Pretoriensis* (8), *N Willmori* (9)

5. *N. Theobaldi.*—Wings, jet black with the costa interrupted by five white spots and an apical spot. Legs, brindled with white scales and a large sub-apical white patch on the femora. Two

and a quarter hind tarsi pure white, then a black band, then a small white one. Palpi, three white bands, apex white, two apical bands equal, a third narrow.

A Nagpur variety which THEOBALD considers may be a distinct species has two-and-a-half hind tarsi white and the tips of the palpi black. India.

6. *N. Maculipalpis* = *A. Jamesii* in *Reports to Royal Society*, STEPHENS and CHRISTOPHERS. Wing, costa black with five white spots. Legs, black spotted with white, last three hind *tarsi* pure white, and apex of next. Palpi, two broad white bands, one apical, a third narrow one towards the base. The rest of the palpi spotted with white. Length, 5·5 mm. India, Africa.

7. *N. Jamesii.*—Costa, four large and two small dark spots. Legs brown, fore femora and tibiae more or less *spotted.* Hind legs, femora and tibiae with an apical white spot, last three tarsi white, and apex of next (Fig. 53). The first tarsal segment of the fore leg has an indistinct median band. Palpi black, with white rings and white apical joint, closely related to *A. Fuliginosus*, but easily distinguished. Length, 3 to 3·5 mm. *Cp. A. Maculipalpis*, length, 5·5 mm.

N. Maculipalpis, v. Indiensis.—Hind legs not quite so banded as in the type. Some variation in wing markings.

8. *N. Pretoriensis.*—Clypeal hairs of larva simple. Palps not mottled, otherwise like *N. Maculipalpis.* The two white apical bands are further apart. Second hind tarsus has a small black patch near its base. Metatarsus, mottled with white and black, and has a broad white

apical band like the first tarsal. The last two hind tarsi only white.

9. *N. Deceptor* (DÖNITZ). Sumatra. *Deceptor* because very like *punctulatus* or *leucophyrus*. Terminal half of *proboscis* white. Terminal half of palpi white, with a narrow black ring at the commencement of the third and fourth joint. The ring around the light end of the second joint, possessed by *A. punctulatus*, is wanting. Legs similarly marked as in *leucophyrus*, excepting the hind legs, which have only a small light spot at the end of the tibia, and not a broad white band.

10. *N. Willmori* (JAMES). Punjab, Kashmir. Wings, four large and three small black areas. Palpi, three white bands, the two terminal ones are equal and broad, the third narrow and basal. Legs, dark brown, thickly speckled with white spots. The last tarsal segment of hind leg pure white.

11. *N. annulipes*. Australia.

12. *N. Masteri*. Australia.

Genus *Cellia*.—Wings densely scaled. Palpi of ♀ densely scaled. Easily recognized by the dense coating of irregular scales

Larvae (*C. Pulcherrimus*).—Antennal hair simple. Clypeal hairs, outer pair branched. Ova, type 2.

Last hind tarsi white:

1. *C. Pulcherrimus*, $3\frac{3}{4}$ white. Punjab.
2. *C. Bigotii*, 3. Chili.
3. *C. Pharoensis*, $1[\frac{1}{3}]$. Egypt, Gambia.
4. *C. Argyrotarsis*, $\frac{1}{2}$. Palpi, three bands, deep black basal band to last tarsus. Acts as a host for *F. nocturna* (VINCENT). West Indies

5. *C. Albipes*, ½. Palpi, two bands. West Indies, Brazil.

Last hind tarsi yellow:

6. *C. Kochii*, 3. Malay.

Last hind tarsus black:

7. *C. Squamosa.* Africa.

Genus *Aldrichia.*—Wings much as in *Myzomyia*, for which genus it was originally mistaken.

1. *Al. Error.*—Resembles *M. Rossii.* Easily distinguished by the abdominal scales. India.

Chapter XVII

THE HABITS OF ANOPHELES

Geographical Distribution

This is as yet far too imperfectly known for a close consideration of the subject to be of much value. We may consider, however, that the *Anophelina* of some portions of Africa and some portions of India are known with a sufficient degree of exactitude to make a comparison of interest. If, however, we take into consideration the distribution in the different parts of the same country, the data are not nearly numerous enough Thus, for instance, *Pyretophorus Jeyporensis* has been recorded, so far, only from Madras (Jeypore); it is almost certain, however, that its distribution must be wider than this. Again, we have at present practically no data of that part of the world intervening between North Africa and India, but it is of interest to note here that so far only two species have been found common to India and Africa, viz., *Nyss. Maculipalpis* and *M. Barbirostris;* many genera are, however, common to Europe, Africa, and India, and among these the species are sometimes closely allied. Thus, *Myzomyia funestus* (Africa) is closely allied, though distinct from *Myzomyia Listoni*. And, further, in the case of these two species we actually know from dissection and not from conjecture that they both

are found naturally conveying malarial sporozoits, and both are associated with areas of high endemicity. The following is a complete list of the known *Anophelina*. Where the same species occurs in more than one country we have indicated the fact by inserting in brackets the number under which it first occurred in the list :—

Europe

1. A. Maculipennis
2. A. Bifurcatus
3. A Nigripes
4. Pyr Superpictus
5. Mym. Hispaniola (and Teneriffe)
6. Myzo. Pseudopictus

Palestine

7. Pyr. Palestinensis
M. Pseudopictus
[1] A. Maculipennis
[4] P. Superpictus

North America

8. A. Maculipennis (? European species)
[3] A. Nigripes
A Pseudo-punctipennis
[2] *A. bifurcatus*
9. A. Punctipennis
10. A. Crucians

South America and West Indies

11. C. Argyrotarsis
12. C. Bigotii
13. Mym. Lutzii
14. Arri. Maculipes
15. Cy. Grabhamii
16. Cyclo. Medio punctatus
17. Steth. Nimba
18. C. Albipes

Africa

19 Mym. funesta
20. Myzo. Paludis
21. Mym. Rhodesiensis
22. C. Squamosus
[4] Pyret. Superpictus (West Coast and Mashonaland)
23. Pyret. Costalis
24. C. Pharoensis (Gambia, Egypt)
25. Mym. Longipalpis
26. Pyret. Marshallii. (Mashonaland)
M. Coustani (Madagascar)
27. Pyret. Cinereus (S. Africa)
28. Mym. Mauritianus
29. Mym. Hebes (E. A. and S. W. A.)
30. Pyret. Merus
31. Nys. Maculipalpis
32. Nys. Pretoriensis
33. Myzo. Barbirostris
34. A. Algeriensis
35. Mym. Impunctus (Egypt)
36. Pyret. Chaudoyei (Algeria)

India

37. Myzo. Nigerrimus
[33] Myzo. Barbirostris
[31] Nysso. Maculipalpis
38. Nysso. Jamesii
39 Nysso. Stephensi
40. Nysso. Theobaldi
41. Nysso. Maculatus
42. Nysso. Willmori
43. Nysso. Karwari
44. Nysso. fuliginosus
45. Mym. Rossii (everywhere)
46. Mym. Listoni
47. Mym Leptomeres
M. Indiensis
48. Mym. Culicifacies
49 Mym. Turkhudi
50 Mym Elegans
51. Myzo. minutus
52. Myzo. varus
53. Ce. Pulcherrima (Punjab)
54 A. Lindesayii (hill species)
55. A. *Immaculatus (very rare)*
56 A. Aitkenii (Goa).
57. A Gigas (hill species)
58. Ald error
P. Jeyporensis

Malaysia

59. *Mym Aconita*
60. Mym. Ludlowii
61. Mym Albirostris
62. Mym. Punctulatus
63. Mym Kochii
[45] Mym Rossii
[46] Mym. Listoni
[52] Mym. varus
[51] Mym minutus
[34] *Myzo. barbirostris*
64 Myzo sinensis (China)
[41] Nysso maculatus
M Tesselatum
P *Minimus (China)*
65. Nysso deceptor
66. Mym umbrosus
67. Mym albotaeniatus
[33] Myzo. plumiger or [64] (Donitz)
[28] Mym. tenebrosus (Donitz)
[50] Mym Leucosphyrus (Donitz)
[44] Nyss Leucopus (Dönitz)
[23] Pyret gracilis (Donitz)
[45] Mym. vagus (Donitz)

Australia

68. *Myzorhynchus Bancroftii*
69 A Stigmaticus
70 N. Annulipes
71 *N Masteri*
72 P Atratipes

Note —*From the Philippines we have M Pseudo-barbirostris* and A Philippinensis, and three unclassified—A *Vincenti* (Tonkin), A *Farauti*, and A *Pursati*, *making a total* of over eighty

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are frozen over. Under these circumstances they grow extremely slowly, if at all.

3. *Hibernation of Eggs.*—There is a certain amount of evidence to shew that eggs can survive for some months in moist earth, exposed to frost, etc. For young larvae have been found in fresh pools in the winter, under conditions that made it unlikely that the eggs had been deposited there on the appearance of water. The resistance of eggs to drying under a tropical sun is, however, practically nil. In the case of the eggs of *Culex fatigans*, Miss EVANS made the following observation in Calcutta :—' Desiccation of the egg-boat for one day was generally sufficient to prevent the larvae from hatching out, though one or two larvae did hatch out of an egg-boat which had been dried (? exposed to the sun) for six days.'

MODE OF DISPERSAL OF ANOPHELINA

There is no evidence existing at present to show that mosquitoes habitually disperse any considerable distance from their breeding-grounds. In fact, the evidence is completely against such a dispersal, and, broadly speaking, the *Anophelina* remain where they were developed, and in the native huts where they find abundant food.

That various accidental modes of distribution occur is equally certain, *e.g.* :—

1. On trains, boats, and even ocean-going steamers, they may be carried long distances, *e.g.*, from West Africa and South America to England, but it remains to be shewn that *Anophelina*, thus introduced, ever effect a permanent habitation, even when the removal by this means is from one portion of the tropics to another.

2. Locally, streams and canals may carry larvae and ova long distances, perhaps miles.

3. *Winds.*—The maximum distance that the *Anophelina* can be carried in this way is quite uncertain. Nearly all of the excessive distances that have been given as possible flights refer to *Culex*. It appears certain, moreover, that the *Anophelina* dislike wind and seek shelter from it.

4. *Trees, Plantations, 'Bush' Jungle.*—These elements undoubtedly hinder the flight of *Anopheles*, and, on the contrary, open spaces promote their diffusion. It is necessary to bear this fact in mind, where a belt of jungle screens off a source of *Anopheles* (larvae), which may find an opportunity of becoming infected later.

'Domestic' and 'Wild' Species of Anopheles

Anopheles are mostly found in association with native dwellings where there is abundance of food (blood). *Anopheles* are also generally abundant where cattle are kept.

Certain species are distinctly 'domestic' in their habits, *e.g.*, *Mym. Rossii*, *Pyr. costalis*, *Nyss. Stephensi*, and others. They are found resting in the daytime in the thatch of huts, and they breed close at hand in the nearest puddle. They may, however, fly up to half a-mile if there are no breeding places closer.

Other species are not peculiar to houses, but are also found breeding in streams and pools in the jungle far from habitations. Such species are *Nyss. Maculatus*, *Nyss Theobaldi*.

The mosquitoes of the genus *Myzorhynchus*, on the contrary, are 'wild' *Anopheles*. They are

only occasionally found in houses. They breed in extensive bodies of water, swamps, rivers, jungle pools, etc. It is *Anopheles* of this type which chiefly frequent one's tent when this is pitched in remote and especially in swampy jungle. The more common species of these wild *Anopheles* are *M. barbirostris*, *M. Sinensis*, *M. paludis*.

Nature of Food

The normal food of the female (domestic) *Anopheles* is blood. In nature they appear to feed every night, the stomach never becoming empty. In *Anopheles* caught under natural conditions, the stomach contents generally shew blood in two or three stages of digestion.

Female *Anopheles* readily drink water, especially if they have been kept for some time in a dry bottle. It seems doubtful whether vegetable juices form an important article of food as appears to be the case with some of the *Culicidae*. Male *Anopheles* can be seen feeding upon banana and other fruit juices, but are, notwithstanding, found dead about the second or third day of captivity.

Under some conditions the females do not feed upon blood, *e.g.*, *A. maculipennis* in England (Theobald).

Bancroft states that *Nyss. Annulipes* will live for a month on dates, but only for three days on bananas.

Time of Feeding

The usual time for feeding of *Anopheles* is after dark, more especially in the early night and before dawn. Occasionally some *Anopheles* may

be found biting in broad daylight, and ANNETT and DUTTON state that *Anopheles* feed readily in certain parts of Nigeria by day. Possibly certain species feed more readily by day than others.

We have ourselves seen on rare occasions *M. Rossii* attempting to feed in the daytime, and GRAY, B.C.A., says 'that *Ce. albipes* when disturbed will bite at *any time of the day or night*.'

On the whole, however, the *Anophelina* are strictly nocturnal in their habits. Nor do they hover around lamps as has been supposed. Of *A. bifurcatus*, BLANCHARD states that it bites fiercely *at dusk, but at night practically not* at all. At dawn, however, it begins again, and it bites at all times in shady places, outhouses, etc.

DISTANCE OF FLIGHT

The *maximum* distance that *Anopheles* can fly requires further study. In questions of flight, the species of mosquito should always be noted. Observations upon the flight of mosquitoes have, so far, been vague and uncritical. With regard to *Anopheles* on ships, it must be borne in mind that *they have not necessarily come from the land* on the night upon which they appear, but may have come on board when the ship was in port or even have been bred on board. In certain villages in India studied by us, *Mym. culicifacies*, *Nyss. Stephensi*, and *Nyss fuliginosus* were always present in abundance, if there were extensive breeding-grounds within quarter-of-a-mile. Where villages were distant half-a-mile from extensive breeding-grounds, they *contained few or no Anopheles*. The only exceptions to this rule were when breeding-places had only recently dried up. In the case of

the above species they undoubtedly fly fairly readily quarter-of-a-mile, but half-a-mile appears to be beyond the normal distance of flight.

RELATION TO COLOUR, ODOUR OF OBJECTS, ETC.

Anyone who, in the tropics, has left his wardrobe open at sunrise and then closed it, and again examined it some time later, will have often observed the well-known fact that, on his white clothes, few or no mosquitoes are resting, but that on his blue serge clothes there may be dozens. He will have noted, too, that outside his mosquito net it is on the shady side that the mosquitoes remain longest, until from here also, they fly away as the fierce sun rises.

He will have noted, too, that *Anopheles* as well as *Culex* have a predilection for certain smells. Old boots and blacking attract them strongly, and the leather of a saddle room is their favourite haunt. *Anopheles*, too, much prefer the odoriferous skin of the native to that of the European, as experiments made by us in Sierra Leone clearly shewed.

NUTTALL and SHIPLEY have made some laboratory experiments on the influence of colour, and find that navy blue is the colour most preferred by *A. maculipennis*, and yellow the one most shunned.

As, however, the *Anophelina* at least are nocturnal in their habits, and prefer biting unclothed portions of the body, the colour of one's clothing will not be much protection. If white or yellow socks can prevent the persistent attacks of *Stegomyia*, it would indeed be a practical boon.

To various trees and plants has been ascribed a repellent effect upon mosquitoes. None of these statements has, so far, borne a critical examination.

Length of Life of Mosquitoes

The length of life of mosquitoes, under suitable conditions, is probably considerable; several weeks to months. In captivity they may, if suitably housed and constantly fed, be kept alive for days, weeks, and even months. A mosquito kept some time in captivity becomes infirm, and readily falls into the water whilst laying its eggs. It also finds difficulty in hanging on to smooth glass, and even though a rough surface is supplied the insect is constantly found on the bottom of the cage resting in a horizontal position. After laying eggs, such infirm mosquitoes generally die the same night. In nature, *Anopheles* certainly remain alive in huts for one or two months and possibly longer. After the drying up of all breeding-places, the winged *Anopheles* do not much diminish in number for several weeks. If the drying up continues, the numbers gradually diminish, but specimens may be caught up to two months or more afterwards.

'Aestivation' of Mosquitoes

In very hot and dry countries, the *Anopheles* which remain through the dry season appear to exhibit some peculiarities in their habits :—

1. Unlike hibernating mosquitoes, they feed regularly and are found full of blood.

2. The ovaries are in the majority large and the ova fully developed.

Chapter XVIII

ANOPHELES—THE OVUM

The Ovum

Anopheles in captivity generally lay their eggs on some floating object, but also upon the surface of the water. When laid on a solid object, and even when laid on the water, the eggs are deposited in a piled up mass. Later, the ova, if on water, often form very regular and beautiful patterns. Brick-red masses of eggs are sometimes laid. These do not develop further.

Observe (i) the arrangement in equilateral triangles and star patterns (Fig. 19).

(ii) The arrangement in rows of eggs lying side by side.

Both patterns are dependent upon the shape of the individual ovum; ova belonging to type 1 forming stars, and ova belonging to type 2, rows.

The number of ova varies, but is usually about one hundred. The size of the ovum varies with different species from about 0·6 to 1·0 mm.

Examination of Anopheles Ova

Anopheles ova (with one exception as yet described) are boat shaped, with an approximately

flat upper surface and a deeply convex lower surface. One end which contains the head of the embryo is blunter and broader than the other. During the act of hatching this end is forced open by the escaping larvae.

1. *The Upper Surface*—Observe that the upper surface is generally granular or tuberculated in appearance. At either extremity it is continuous with the pointed ends of the ovum, and in this position there are usually several small polygonal areas. The width of the upper surface and the extent to which it is encroached upon by the floats varies in different species.

2. *The Lower Surface.*—The lower surface is generally smooth and dark grey. In damaged ova a silvery membrane will be seen partly detached, shewing a deep shiny-black surface beneath. The silvery membrane is the outer covering of the egg, and formed by the layer of follicular epithelium (Fig. 39). In some species the lower surface is marked with silvery lines forming a reticular pattern.

3. *The Floats.*—Occupying about the middle third of the side of the ovum is a remarkable structure—the float. This consists of a very delicate membrane continuous with the chitinous cuticle covering the whole ovum and containing air cells.

The floats are generally oval in shape and shew regular transverse corrugations. The shape and position of the floats vary considerably in the different species.

4. *The Frill.*—Around the margin of the upper surface (forming the gunwale of the boat) there is in some species a gleaming white frill-like

structure. This is striated in appearance, but portions of it may (in some species) be free from striations. In other species the appearance is rather that of a white striated rim. In all species of *Anopheles* ova yet described, *a striated* frill or rim is present. The width and extent of the frill vary in different species.

Three very distinct types of ovum have been seen by us—

Type 1.—Ova have the upper surface very narrow, with the lateral *floats not touching the* margin (Fig. 54 : 1).

The species with ova of this type are—

M. barbirostris	*M. culicifacies*
M. Sinensis	*M. Listoni*

sub-sp. *nigerrimus*

Type 2.—Ova having a more or less broad upper surface, with the lateral floats touching the margin (Fig. 54 : 2, 3, 4, and 6).

Species having ova of this type are—

M. Rossii	*N. fuliginosus*
Ce. pulcherrimus	*N. Stephensi*

Type 3.—Ova with no floats, and with upper surface rudimentary (Fig. 54 : 5).

One species only as yet described has ova of this type, viz. :—

M. Turkhudi

Species having ova of the first type have in all cases been species breeding in either open natural waters or running streams.

Species with ova of the second type are in general found breeding in pools.

The only ova as yet systematically described are those of the Indian *Anopheles*. Further observations will probably add further types to the above.

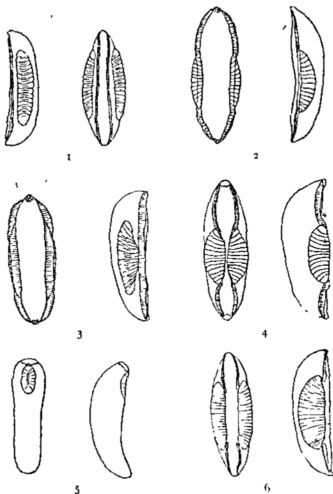


Fig 54 *Ova of Anophelina*
1. *M. Culicifacies* 2 *C Pulcherrimus* 3 *M Rossii*
4. *N. Stephensi* 5 *M Turkhudi* 6 *N Maculipalpis*

Within each type great variation usually exists in the different species. The following are the most notable variations found :—

1. *The Frill.*—The width. The continuity of the frill around the whole of the margin of the upper surface or its replacement in the middle third by the floats. The extent of striation of the frill. The presence of a striated rim only.

2. *The Floats.*—The position, placed forwards and encroaching on the upper surface, or laterally situated. The shape, oval, globular, or scallop-shell.

3. *The Lower Surface.*—Whether ornamented or not with silvery reticulated pattern.

The following is a brief résumé of the characters of the ova of *Anopheles*, as far as these have been described :—

Type 1. *M. Sinensis*, sub-sp. *nigerrimus*

Ovum.—Upper surface very narrow. Floats do not touch margin of upper surface. Lower surface of ovum ornamented with polygonal markings.

M. barbirostris

Ovum.—Upper surface very narrow. Floats do not touch margin of upper surface. Lower surface of ovum ornamented with polygonal markings.

M. culicifacies

Ovum.—Upper surface very narrow. Floats do not touch margin of upper surface. Lower surface not ornamented. A short but distinct fringe is continued around margin of upper surface.

M. Listoni

Ovum.—Upper surface very narrow. Floats do not touch margin of upper surface. Lower surface not ornamented. A small fringe passes around margin of upper surface.

Type 2. *M. Rossii*

Ovum.—Upper surface broad. Fringe very well developed and striated throughout whole length. Floats scallop-shell shape and touch margin of anterior surface. Lower surface not ornamented.

Ce. pulcherrimus

Ovum.—Upper surface broad. Floats touch margin of upper surface. Fringe well developed around margin of upper surface. Striations are not present in that portion of the fringe lying over the floats. Lower surface not ornamented.

N. fuliginosus

Ovum. Upper surface moderately broad. Floats touch margin of upper surface. Floats long and narrow. Fringe around upper surface only indicated by white border. Lower surface not ornamented.

N. Maculipalpis

Ovum. The upper surface is rather narrow. The floats are rather short and oval, and are placed far forwards as in the ovum of *N. Stephensi*, though less markedly so. The fringe is fairly developed, but is not continued over the floats.

N. Stephensi

Ovum.—Upper surface broad, except in central portion where encroached upon by floats. Floats placed on margin of upper surface so that they touch, or nearly touch, one another in middle line. Floats short and almost globular. Fringe not well developed. Lower surface not ornamented.

N. Theobaldi

Ovum.—As the females of this species have only been very occasionally caught by us in houses, we have not been able to describe the ovum as deposited by the insect. Fully developed ova removed from a bred specimen showed, however, that the ovum resembled that of *N. Maculipalpis.* The floats were rather short and situated far forwards as in *N. Stephensi.* The fringe is fairly developed, but does not pass over the floats.

Type 3. *A. Turkhudi*

A. Turkhudi is a very aberrant type, so far as the ovum and larva are concerned. Both the ovum and larva approach to the characters of the *Culex* ovum and larva. The eggs were laid upon a floating object. When placed upon water they sank. They were laid in the heaped-up manner sometimes adopted by *Anopheles,* especially *M. Rossii* and *N. maculipalpis.* The chief characters of the ovum are:—

1. No separation of an upper surface as in all other *Anopheles* ova. At the thicker end of the ovum there is an oval area about a quarter the length of the whole egg. This is glistening

white and striated, and probably represents the upper surface of other *Anopheles* ova.

2. There are no floats or any markings representing them.

3. There is a pale area at the thicker end of the egg with a scalloped edge.

4. The ovum is otherwise without markings.

It is obvious that the characters of the ovum are of considerable importance in the classification of *Anopheles*, and every care should be taken to describe these in as great detail as possible.

In making drawings of the ova of *Anopheles*, it is convenient to use an eyepiece micrometer, the width of the frill and of the upper surface and the length of the floats as compared with the length of the ovum may all be readily and accurately noted.

To Mount Ova

No thoroughly satisfactory method is known to us, but although imperfect, any of the following methods will give specimens in which some, at least, of the ova preserve most of their characteristics.

1. Place the eggs on a slide which has been made slightly sticky with balsam, and then mount them in a drop of balsam and place a cover-glass over them.

2. Mount in two per cent. formalin solution and ring the coverglass with balsam or shellac.

3. Mount in glycerine and ring the specimen.

4. Mount in a drop of cedar-wood oil

3. *The Eyes.* The eyes are situated laterally, and can be seen both from the dorsal and ventral surface. Their size and appearance vary with the age of the larva. In the full grown larva a crescentic compound eye is seen on either side, and behind this a single pigment mass (simple eye). The compound eye is absent in the first stages, and becomes more prominent as the larva approaches maturity.

4. *The Mouth Parts.*

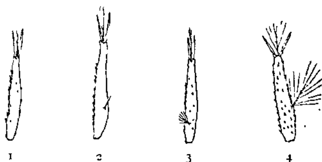


Fig. 55. *Lateral Hairs of Antennae*
1. *M. Rossii.* 2. *N. Stephensi.* 3. *A Lindesayii* 4. *M. Nigerrimus*

(*a*) Two very conspicuous bodies resembling somewhat shaving brushes are protruded or withdrawn under the overhanging clypeus. These are the feeding-brushes, and are employed in collecting the minute food particles on which the larva feeds.

(*b*) On either side of the mouth is a broad blade-like structure carrying several leaflets and some hairs (maxillary palps).

(*c*) Below the feeding brushes, and not so easily visible, are two stout bodies with comb-like projections (mandibles).

(*d*) In the middle inferior line lies a conical toothed structure. The *under lip* of Meinert (Fig. 23).

(*e*) In the fully-grown larva a snout-like process covered with short hairs projects forwards in the middle line between the brushes.

The front portion of the head projects between the antennae as a semi-circular smooth area. In front of this is a protrusion overhanging the base of the brushes (the clypeus).

5. *The Clypeal Hairs.*—These are four or six in number. *Two spring from the extreme front* of the clypeus near the middle line; two from the outer corner of the clypeus immediately over the feeding-brushes, and two usually very small and not always present behind the origin of the others.

The clypeal hairs are best seen when the feeding brushes are retracted. They must not be confounded with certain other hairs on the larval head. These are:—

(i) Six large branched hairs arising from the prominence lying between the bases of the antennae.

(ii) Four similar branched hairs, but smaller, situated further back (Nuttall and Shipley).

The hairs exhibit great variation in different species, but are quite constant in the one species. A minute description of these hairs is of great importance in describing the specific characters of the larva.

Clypeal Hairs of Larvae:—

(i) The four anterior hairs may be quite simple and unbranched. *Anopheles* having larvae of this type are *M. Rossii, N. Stephensi, M. culicifacies, M. Listoni, M. Turkhudi* (all Indian species).

(ii) All four anterior hairs may shew small lateral branches. *P. Jeyporensis.*

In *A. maculipennis* all four hairs are branched, the outer pair form distinct tufts.

(iii) The outer pair may be markedly branched (from six to twelve hairs or more arising from near the point of origin), *e.g., Ce. pulcherrimus.*

(iv) The outer pair may be developed into a close tuft (cockade), *e.g., M. barbirostris.*

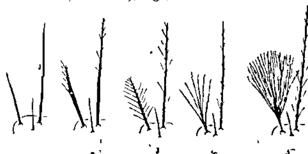


Fig. 56 Clypeal Hairs of Larvae
1. *M. Rossii, N. Stephensi, M. Culicifacies, M. Listoni* 2. *N. Maculipalpis* 3. *P. Jeyporensis.* 4. *Ce. Pulcherrimus* 5. *M. Sinensis, M. Barbirostris*

The two hairs situated behind these may, instead of being very short and inconspicuous, be long and prominent, *e.g., M. Turkhudi.*

6. *The Thorax.*—The thorax in the adult larva is large and globular. In the young larva it is not so broad as the head, but becomes proportionately larger as the larva advances in age.

Numerous hairs arise from the front and sides of the thorax. None of these vary perceptibly in different species. A number of large hairs arising from papillae on the lower surface are capable of being used almost as a means of progression when the larva is in very shallow water.

(*a*) Observe on the dorsum of the thorax a short but extremely stout and strong hair, unlike the others, projecting outwards and forwards.

(*b*) A flap-like body may, with careful focussing, be seen lying at the base of the most anterior hairs on either side.

(*c*) In some species of *Anopheles* a single pair of palmate hairs, similar to those on the abdominal segments, are found upon the thorax. In others they are rudimentary or absent. The presence of well-developed palmate hairs on the thorax is of specific importance.

(i) It is well developed and functionally active in *M. culicifacies*, *M. Listoni*, *P. Jeyporensis*.

(ii) It is rudimentary or absent in all other larvae as yet described.

7. *The Abdomen.* —The first seven segments are very similar in shape. The eighth carries the opening of the air-tube, and the ninth some curious papillae and large hairs.

Each of the first two segments carries on each side a pair of long feathered hairs. The third carries a single similar hair. On the other segments there are much smaller and unfeathered hairs. On all the segments there are groups of small hairs which do not appear to vary and which are not of sufficient importance to describe here. None of the above structures appear to vary in different species.

Examination of Palmate Hairs.—The most important appendages of the abdominal segments are certain small fan or palm leaf-shaped hairs attached by a short stalk to the outer dorsal portions of certain of the segments (Fig. 57A). The number of segments bearing well-developed palmate hairs varies in different species.

Place the larva under a coverglass in a drop of water or use a permanently mounted larva.

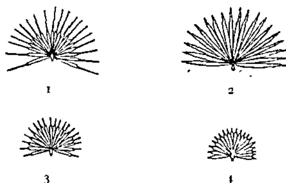


Fig. 57A. *Palmate Hairs of Larvae:*
1. *M Rossii.* 2. *M. Nigerrimus.* 3. *M. Listoni* 4. *N. Maculata*

1. Determine the number of segments which carry distinct and large palmate hairs and those carrying ill-developed ones.
2. Carefully cut out with a needle two or three central abdominal segments. Crush these.

The following arrangement of these hairs is found in Indian species of larvae, the only larvae as yet systematically described.

1. Fully developed hairs on all segments (one to seven) and on the thorax.

P. Jeyporensis
M. Listoni
M. culicifacies

2. Fully developed hairs on the second to seventh, or third to seventh segments. Rudimentary hairs on the second or even first abdominal segments and on the thorax.

N. Stephensi
N. maculata
N. Theobaldi

3. Palmate hairs confined to the third, fourth, fifth, sixth, and seventh segments.

M. Sinensis
M. barbirostris
A. maculipennis (NUTTALL and SHIPLEY)

4. Palmate hairs confined to the fourth, fifth, and sixth segments.

M. Turkhudi

The Leaflets —In the well grown larvae each palmate hair consists, as a rule, of nineteen or twenty leaflets arising close together from a short stalk, and forming a semi-circular fan. When collapsed, as is the case when the larvae is beneath the surface, these hairs are inconspicuous. When, however, the larvae takes up its characteristic attitude at the surface of the water, these spread out fan-like, and are very striking objects under the microscope. In the freshly hatched larva, the separate leaflets appear to be folded together, so that the hair has the appearance of a single lanceolate structure. About the third day, the hairs are seen with seven to eight uniformly lanceolate leaves. Very soon after this, they take on

the characters seen in the hairs of the mature larva.

In the mature larva the leaflets shew much variation in the different species. In most species, the leaflets terminate rather suddenly in a number of jagged points or notches, whilst the central portion continues as a more or less fine filament.



Fig. 57B. *Leaflets of Palmate Hairs*
1. *M. Sinensis, M. Barbirostris* 2 *A Lindesayii*
3 *N Theobaldi, N. Stephensi.* 4. *M Listoni, M. Culicifacies*
5 *M. Rossii.* 6. *M. Turkhudi*

The character of the notching and the relative length of the filament to the leaflet are of specific importance. The following types of leaflets are known :—

1. The *leaflets* are *unbrokenly* lanceolate in shape, with saw-like notches along the edge of the outer half. There is no distinct terminal filament.

M. Sinensis
M. barbirostris

2. The filament is long and filamentous.

M. Rossii
M. culicifacies
M. Listoni
N. fuliginosus

Further differences are seen in the case of most of the above species. In *M. Rossii* the filament is as long as the leaflet, and there is scarcely any notching where the two join. In *N. Theobaldi*, the notching is well marked (Fig. 57B).

3. *The filament is very short, a mere spike-like process.* *N. Stephensi*
N. maculata
N. Theobaldi
N. maculipalpis

The Stigmatic Syphon.—The eighth segment bears the stigmatic opening. This is a large quadrilateral space, with hard comb like chitinous processes on either side. These have the teeth projecting backwards, and are capable of being approximated so as to obliterate the cavity. Into *the anterior portion of the space, under cover of a* lip-like process, the two main air tubes open.

The ninth segment is cylindrical in shape, and is chiefly notable from the fact that it carries four large transparent papillae well supplied with air tubes and certain long curved hairs. Of the hairs one series projects downwards so as to resemble a *rudder. The others project posteriorly. There* does not appear to be much variation in the different species.

EXAMINATION OF THE LARVA

1. Some features, *e g.*, feeding, are conveniently studied by placing the larvae in a drop of water in a watch glass.

2. For examining under a high power, the activity of the larva must be restrained by a cover-glass.

3. Permanent preparations may be made at once by placing in strong formalin, then alcohol, then oil of cloves, then balsam (*vide* p. 250).

4. Beautiful preparations of the palmate hairs, etc., are got by mounting the larval skeleton thrown off at the time of pupation.

Pupation

Just before this process the larva becomes quieter. The attitude also frequently alters, becoming a hanging one, somewhat like that of a *Culex* larva.

In this condition larvae are very readily killed by agitating the water (and it is difficult to carry larvae in this stage without killing them).

The change into the nymph is very sudden. A few rapid motions and the larval skin is cast off, leaving the characteristic nymph.

The Nympha

This stage in the tropics usually lasts about forty-eight hours. When first the larval coat is cast the lymph is light in colour, and may be readily overlooked. Later, the nymph becomes darker, and towards the end and immediately prior to the emergence of the imago, *silvery patches* due to collections of air are seen beneath the cuticle.

Pupae taken out of the water and kept on moist blotting-paper will still develop into winged insects (Nuttall and Shipley). For the distinction between *Anopheles*, *Culex*, and other nymphae, *vide* p. 89. No differences have been described between the nymphae of the different species of *Anopheles*.

Chapter XX

THE BREEDING-PLACES OF ANOPHELES

Breeding-Places of Anopheles

Directions for the collection of *Anopheles* larvae have already been given.

It is a matter of considerable importance to know what species breed in any particular situation. Larvae should be sought for in the most diverse situations and, after being examined and described, be allowed to hatch out. New species of *Anopheles* are often obtained in this way. It is the case in India, and almost certainly will be found to be so in other countries, that certain kinds of breeding-places are preferred by certain species. A collection of larvae made from shallow puddles will be found to yield quite a different set of species to one made from a streamlet or pool full of vegetation, even though close to the puddles (Fig 58)

The following tabular statement gives the more common situations of *Anopheles* breeding-places and the species, as far as known, found in each. It is obvious that a great deal of work yet remains to be done

1 Foul puddles near habitations	*M* Rossii
2. Clean puddles without much alga and often turbid with suspended matter —	
(a) Pits and puddles near houses	M Rossii P costalis

(b) Roadside puddles	N. maculata P. costalis
(c) Cattle footmarks	N. Stephensi
(d) Shallow, muddy sheets of water	M. Rossii
(e) Pools in sandy river beds (f) Large pools in quarries, etc.	M. culicifacies M. Turkhudi
3. Puddles and pools with much alga. Common in stream beds, water trickling over rocks	N. Fuliginosus N. maculata
4. Earthenware vessels, empty paraffin tins, boats, water barrels	N. Stephensi P. costalis
5 Wells, springs	N. Stephensi P. costalis A. Lindesayii
6 Swamps.	
(a) Deep water with much aquatic vegetation	M. Sinensis M. barbirostris M. paludis
(b) Rice-fields, wet cultivation of all kinds	M. Rossii P. Jeyporensis N. maculata N. *maculipalpis* A. Lindesayii
7. Running water	
(a) Swiftly flowing streams	M Listoni
(b) Sluggish irrigation channels, ditches, muddy trickles, edges of rivers	M. Funesta M. culicifacies N. maculipalpis
(c) With much weed and alga	M. Sinensis M. barbirostris N. fuliginosus N. maculata N. Theobaldi
(d) Stony and shallow	M. culicifacies N. Theobaldi M. Turkhudi

8. Lakes with weedy margins	N. fuliginosus A. Lindesayii
9. Hill species	N. maculata A. gigas

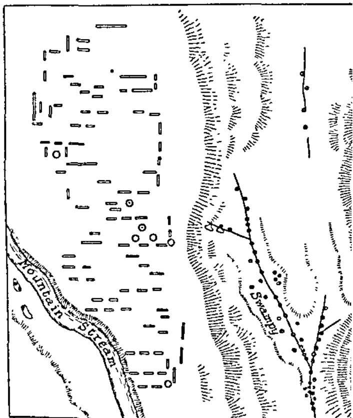


Fig. 58 Portion of Coolie Lines on a Tea Plantation to shew different breeding-places of M. Rossii, M. Listoni, and N. Maculata

○ = *M. Rossii* ● = *M. Listoni and N. Maculata*

(b) Roadside puddles	N. maculata P. costalis
(c) Cattle footmarks	N. Stephensi
(d) Shallow, muddy sheets of water	M. Rossii
(e) Pools in sandy river beds (f) Large pools in quarries, etc.	M. culicifacies M. Turkhudi
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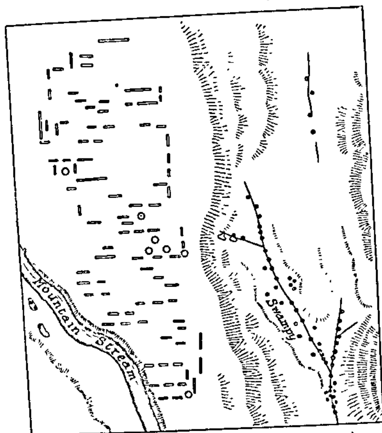


Fig. 58 Portion of Coolie Lines on a Tea Plantation to shew different breeding places of M Rossii, M Listoni, and N Maculata

○ = *M Rossii* ● = *M Listoni and N Maculata*

Chapter XXI

THE IDENTIFICATION OF ANOPHELES LARVAE

1. *Naked Eye Characters.*—Some larvae may be identified by the naked eye. The distinction, however, between most species is insufficient to allow of separation by this means.

2. Observe that the colour of larvae is not dependent on species but on the nature of the food, amount of light they have been exposed to in nature, the colour of the water, and other general conditions.

3. The most distinctive of *Anopheles* larvae are those of *M. Sinensis* and *M. barbirostris*. These are very large larvae, most frequently black, or black speckled with white, but also brown or vivid green in colour. One of their characteristics is a peculiar 'stick-like' appearance, and the assumption of a bent or contorted attitude.

The larvae of *M. Turkhudi* can be detected by their attitude, which is almost *Culex*-like. Larvae about to change into nymphae, however, also frequently adopt this position.

Naked eye examination always requires verification by the microscope.

(A) Larvae may be bred from ova deposited by females of a known species. To successfully accomplish this requires a good deal of care.

1. Remove the paper upon which the ova have been laid (p. 96), and place in a small bottle containing some filtered fresh water from a pool or rain puddle.

2. *Place* in a good light, but take care that the sun, by the focussing action of the glass, does not heat the water, otherwise the larvae will be killed.

3. When the larvae are hatched, transfer them (after a day or two) to a larger vessel of fresh water containing some weed. When the fresh natural appearance of the water disappears, more fresh water from a pool should be added.

4. By keeping larvae in a not too porous earthenware vessel, they may be placed with impunity all day in the direct sun. It is necessary, however, to watch carefully, to guard against desiccation and consequent death of the larvae.

*Larvae kept in flat, partially glazed earthen*ware vessels, with a certain amount of mud, and placed in the sun, develop more quickly than those kept in bottles.

It is of course necessary to make certain that foreign ova or young larvae are not introduced with the fresh water

Some larvae are exceedingly difficult to rear artificially, notably those of *M. barbirostris* and *M. Sinensis*. They remain for long periods without perceptibly increasing in size, and frequently die.

(B) An alternative and less tedious way is to examine nearly adult larvae found in nature, and to observe, after accurately noting the larval characters, what species of *Anopheles* eventually hatches out.

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(B) An alternative and less tedious way is to examine nearly adult larvae found in nature, and to observe, after accurately noting the larval characters, what species of *Anopheles* eventually hatches out.

By examining the larva on a slide without a coverglass, the main characters may be noted without in any way damaging the larva, which later becomes a nymph and eventually an imago. As a rule, however, many specimens of the same species are found together. By a preliminary examination, larvae shewing the same characters may be sorted out, and some specimens afterwards mounted and subjected to a more detailed examination, whilst the rest are allowed to hatch out in due course.

The characteristics of the larvae which are of specific importance are, as we have seen—

1. The antennae.
2. The clypeal hairs.
3. The leaflets of the palmate hairs.
4. The segments carrying palmate hairs.

By means of these characters most species of *Anopheles* larvae can be identified. So far as Indian *Anopheles* are concerned, the following characters hold good :—

Type 1.—Larvae with the external pair of clypeal hairs converted into a cockade-like tuft (Fig. 56).

Species having larvae of this type are—

M. barbirostris
M. Sinensis, sub-sp. *nigerrimus*

Larvae of this type also have a large branched hair upon the antenna, and the leaflets of the palmate hairs differ markedly from all other larvae (Fig. 57B).

Type 2.—Larvae with the external frontal

hairs branched but not developed into tufts (Fig. 56). *N. fuliginosus*
Ce. pulcherrimus

Type 3.—Larvae with the external pair of frontal hairs simple and unbranched, and with palmate hairs on every abdominal segment and on the thorax (Fig. 56).

M. culicifacies
M. Listoni

Type 4.—Larvae with the external pair of frontal hairs simple and unbranched, but with no developed palmate hairs on thorax or first abdominal segment (Fig. 56).

M. Rossii
N. Stephensi

Type 5.—Larvae with two large additional hairs placed behind those already mentioned. Also with first three abdominal segments free from palmate hairs. *M. Turkhudi*

Larva of M. barbirostris.—Antenna with large branched hair. External pair of frontal hairs developed into cockades. Palmate hairs on second to seventh abdominal segments. Leaflets of palmate hairs lanceolate in shape and deeply serrated in outer half. Head of larva without pigmented markings.

Larva of M. Sinensis.—Antenna with large branched hair. External pair of frontal hairs developed into cockades. Palmate hairs (?) Leaflets of palmate hairs lanceolate with serrations in outer half. Head of larva without pigmented markings.

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4. The segments carrying palmate hairs.

By means of these characters most species of *Anopheles* larvae can be identified. So far as Indian *Anopheles* are concerned, the following characters hold good :—

Type 1.—Larvae with the external pair of clypeal hairs converted into a cockade-like tuft (Fig. 56).

Species having larvae of this type are—

M. barbirostris

M. Sinensis, sub-sp. *nigerrimus*

Larvae of this type also have a large branched hair upon the antenna, and the leaflets of the palmate hairs differ markedly from all other larvae (Fig. 57B).

Type 2.—Larvae with the external frontal

hairs branched but not developed into tufts (Fig. 56). *N. fuliginosus*
Ce. pulcherrimus

Type 3.—Larvae with the external pair of frontal hairs simple and unbranched, and with palmate hairs on every abdominal segment and on the thorax (Fig. 56).

M. culicifacies
M. Listoni

Type 4.—Larvae with the external pair of frontal hairs simple and unbranched, but with no developed palmate hairs on thorax or first abdominal segment (Fig. 56)

M. Rossii
N. Stephensi

Type 5.—Larvae with two large additional hairs placed behind those already mentioned. Also with first three abdominal segments free from palmate hairs. *M. Turkhudi*

Larva of M. barbirostris.—Antenna with large branched hair. External pair of frontal hairs developed into cockades. Palmate hairs on second to seventh abdominal segments. Leaflets of palmate hairs lanceolate in shape and deeply serrated in outer half. Head of larva without pigmented markings.

Larva of M. Sinensis.—Antenna with large branched hair. External pair of frontal hairs developed into cockades. Palmate hairs (?). Leaflets of palmate hairs lanceolate with serrations in outer half. Head of larva without pigmented markings.

The habits of both these species, *M. barbirostris* and *M. Sinensis*, sub-sp. *nigerrimus*, are very similar.

The larvae are found in water with much aquatic vegetation—rivers, lakes, ponds, and swamps. They are only caught singly, but are generally widespread in their occurrence where large bodies of water are present.

Larva of N. fuliginosus.—Antenna without large branched hair. External pair of frontal hairs branched (branches usually six in number). Palmate hairs on second to seventh abdominal segments. Leaflets of palmate hairs with very marked 'shoulder' at origin of terminal filament. Terminal filament from one-half to two-thirds the length of basal portion. Head of larva with distinctive markings.

Larva of M. culicifacies.—Antenna without large branched hair. Frontal hairs all unbranched. Palmate hairs on first to seventh abdominal segments, and a pair of fairly developed ones upon the thorax. Palmate hairs with terminal filament nearly as long as basal portion. Head with markings.

Larva of M. Listoni.—Antenna without large branched hair. Frontal hairs simple. Palmate hairs on all segments, and very well developed pair on thorax. The palmate hairs in this species are very large. The terminal filament is nearly as long as basal portion.

Larva of Ce. pulcherrimus.—Antenna without large branched hair. Outer pair of frontal hairs branched (six branches). Palmate hairs on second to seventh abdominal segments. Filament of palmate hair nearly as long as basal portion. Head markings present.

Nature of breeding-place unknown.

Larva of M. Rossii.—Antenna without large branched hair. Frontal hairs unbranched. Palmate hairs second to seventh abdominal segments. Terminal filament of palmate hair very long; *often longer than basal portion.* The 'shoulder' at the origin of the filament is very slightly marked. There are markings upon the head (Fig. 57A).

Breeds nearly always in small pools near houses. *These pools are frequently foul and* nearly always muddy The female lays her eggs very readily in captivity.

Larva of N. maculipalpis.—Antenna without large branched lateral hair. Frontal hairs are *peculiar and show a condition intermediate* between the branched hairs of *M. barbirostris*, *N. fuliginosus*, and the unbranched hairs of *M. Rossii* and other species (Fig. 56). Palmate hairs on second to seventh segments. Leaflets of palmate hairs have very short filaments. The notching at the termination of the leaflet is not so marked as in *N. Theobaldi.*

Larva of N. Theobaldi (GILES).—Antenna without large branched lateral hair. Frontal hairs unbranched. Palmate hairs on second to seventh segments. Leaflets of palmate hairs have very short filaments. There are marked notches at the ending of the leaflet in the filament (Fig. 57B).

The larvae of this species frequent especially sluggish streams with much growth of alga. They were found by us in Nagpur in association with *N. fuliginosus*, *M. barbirostris*, and *M. Listoni*

The habits of both these species, *M. barbirostris* and *M. Sinensis*, sub-sp. *nigerrimus*, are very similar.

The larvae are found in water with much aquatic vegetation—rivers, lakes, ponds, and swamps. *They are only caught singly*, but are generally widespread in their occurrence where large bodies of water are present.

Larva of N. fuliginosus.—Antenna without large branched hair. External pair of frontal hairs branched (branches usually six in number). Palmate hairs on second to seventh abdominal segments. *Leaflets of palmate hairs with very* marked 'shoulder' at origin of terminal filament. Terminal filament from one-half to two-thirds the length of basal portion. Head of larva with distinctive markings.

Larva of M. culicifacies.—Antenna without large branched hair. Frontal hairs all unbranched. *Palmate hairs on first to seventh abdominal* segments, and a pair of fairly developed ones upon the thorax. Palmate hairs with terminal filament nearly as long as basal portion. Head with markings.

Larva of M. Listoni.—Antenna without large branched hair. Frontal hairs simple. Palmate *hairs on all segments, and very well developed* pair on thorax. The palmate hairs in this species are very large. The terminal filament is nearly as long as basal portion.

Larva of Ce. pulcherrimus.—Antenna without large branched hair. Outer pair of frontal hairs branched (six branches). Palmate hairs on second to seventh abdominal segments. Filament of palmate hair nearly as long as basal portion. Head markings present.

Nature of breeding-place unknown.

Larva of M. Rossii.—Antenna without large branched hair. Frontal hairs unbranched. Palmate hairs second to seventh abdominal segments. Terminal filament of palmate hair very long; *often longer than basal portion.* The 'shoulder' at the origin of the filament is very slightly marked. There are markings upon the head (Fig. 57A).

Breeds nearly always in small pools near houses. These pools are frequently foul and nearly always muddy. The female lays her eggs very readily in captivity.

Larva of N. maculipalpis.—Antenna without large branched lateral hair. Frontal hairs are peculiar and show a condition intermediate between the branched hairs of *M. barbirostris*, *N. fuliginosus*, and the unbranched hairs of *M. Rossii* and other species (Fig 56). Palmate hairs on second to seventh segments. Leaflets of palmate hairs have very short filaments. The notching at the termination of the leaflet is not so marked as in *N. Theobaldi*.

Larva of N. Theobaldi (GILES).—Antenna without large branched lateral hair. Frontal hairs unbranched. Palmate hairs on second to seventh segments Leaflets of palmate hairs have very short filaments. There are marked notches at the ending of the leaflet in the filament (Fig. 57B).

The larvae of this species frequent especially sluggish streams with much growth of alga. They were found by us in Nagpur in association with *N. fuliginosus*, *M. barbirostris*, and *M. Listoni*.

Larva of M. Turkhudi.—The larva is *Culex*-like in some of its characters, though undoubtedly much more nearly related to the *Anopheles* type.

The full-grown larva is distinguished by the adoption of the slightly hanging attitude. The chief characters of the larva are :—

1. Two large additional frontal hairs are developed, which reach as far forward as the longest of the hairs described in other larvae.

2. The shape of the head differs from that of the ordinary *Anopheles* larva.

3. The palmate hairs are only represented on two or three abdominal segments, namely, the fourth, fifth, and sixth. They are absent on the first three abdominal segments.

4. The palmate hairs are small and poorly developed. The leaflets are irregular and the terminal filament blunt.

This species must be looked upon as a form which in its egg and larval stages has lost many of the characteristics of *Anopheles* eggs and larvae, and has approached in these stages the characters of the eggs and larvae of *Culex*.

Classification of Indian Anopheles According to Larval Characteristics*

			Species	Characteristics
Antenna with lateral branched hair	Tufted frontal hairs		M. Sinensis	Leaflets of palmate hairs lanceolate and serrated
			M. barbirostris	Ditto
	Simple frontal hairs		A Lindesayi	Leaflets of palmate hairs shewing regular and deep notching (Fig. 57B)
Antennae without branched lateral hair	Simple unbranched clypeal hairs	Filaments of leaflets long	M Rossii	Palmate hairs well developed on third to seventh segments.
			M. Culicifacies	Palmate hairs on all segments and on thorax
			M Listoni	Palmate hairs very large on all segments and on thorax
		Filaments of leaflets short	N Stephensi	
			N maculata	
			N Theobaldi	
	Feathered clypeal hairs	Filaments short	N maculipalpis	
		Filaments long	P. Jeyporensis	Palmate hairs very large on all segments and thorax
	Branched clypeal hairs		N fuliginosus	Filaments of leaflets long
			Ce Pulcherrimus	
	Two posterior hairs large and conspicuous		M Turkhudi	Palmate hairs on fourth, fifth, and sixth segments only; filaments short, leaflets rudimentary

* The Larvae of the remaining Indian species are, so far, undescribed.

To Mount Larvae

1. Place a drop of formalin in a hollow ground slide. The drop must be just sufficient to fill the cell when the coverglass is in position.

By means of a pipette or spoon take up a larva and, removing the excess of water, allow the larva to float off into the drop of formalin.

Place the coverglass in position, avoiding air bubbles, and ring with Canada balsam, etc.

It is important that no air bubbles are included, as a white deposit forms around them.

If too much formalin has been added, the excess must be carefully removed before ringing. If hollow ground slides are not available, a ring of balsam may be made on the slide and allowed to become somewhat hard. Fill the cavity with formalin, place the larva therein, and cover carefully with a coverglass. Avoid excess of fluid or air bubbles. It is best to allow the Canada balsam to be just soft enough to stick to the coverglass.

Larvae mounted in this way retain their characters very well, and the clypeal and palmate hairs can be examined with ease.

2. *To Mount in Balsam.*—If placed in alcohol, oil of cloves or xylol, and balsam in the ordinary way, the shrinkage of the soft parts and even of the hairs is very great.

On no account touch the larvae with forceps, and only occasionally, and with the utmost care, with a needle point.

Place a number of larvae in a covered watch-glass containing formalin. Leave for twenty-four hours at least.

Lift each larva carefully by means of a strip of cigarette paper. Drain off the excess of formalin, and place with the greatest care in absolute alcohol. Allow the specimen to remain for at least ten minutes in alcohol.

Remove with cigarette paper to a watch glass containing oil of cloves. With cigarette paper transfer to a slide. Remove excess of oil of cloves, mount in a large drop of balsam, taking care that the dorsum of the larva is upwards.

If great care is taken not to detach the hairs by handling, the larval characters are beautifully displayed in this way.

It is well-known in a general way that in one country malaria is more intense than in another, but here we have a means of exactly measuring this difference, and, moreover, in the different parts of any particular district. We may illustrate this by the differences we found in Bengal in an extent of country where, as far as we could judge, the climatic conditions were practically identical, yet we find in the environs of Calcutta the endemic index is 0, while in the Duars (at the foot of the Himalayas) it is as high as seventy-two (Fig. 59). We found, however, that there was one important matter in which the Duars differed from Calcutta, and that was in its *Anopheles* fauna. Whereas in Calcutta *M. Rossii* was the predominant species, in the Duars *M. Listoni* was the commonest *Anopheles*.

Again, in the Jeypore district (Madras), we had a district of uniformly high endemic index, fifty to one hundred, and here we found an *Anopheles*, *P. Jeyporensis*, which we had not encountered elsewhere, so that the view seemed tenable that the high endemicity of these districts was dependent on their special *Anopheles* fauna. To test to what extent species was concerned in determining endemicity, we then made use of another more exact method, viz., determining by dissection whether any difference occurred amongst the different species in the percentage of infected specimens: we were able to carry this out in the case of *M. Rossii* and *M. culicifacies*. We caught these species in the same huts in the same villages at the same time, and determined by actual dissection the percentage of glands infected with sporozoits. The results were most striking, and

fully confirmed our previous idea, based on more general considerations of the importance of species. They were as follows :—

I. MIAN MIR (PUNJAB).

	Number dissected	Number with sporozoits	Percentage
M. Culicifacies -	259	12	4 6
M. Rossii - -	495	0	0

II. ENNUR (MADRAS)

	Number dissected	Number with sporozoits	Percentage
M. Culicifacies -	69	6	8 6
M. Rossii - -	364	0	0

Undoubtedly then, under natural conditions, the species is here a very important factor.

Again, under artificial conditions (feeding experiments), we found that there was a difference in the number of zygotes found in the stomach as the result of feeding.

The species which appeared to be most active were :—

M. culicifacies
N. Stephensi
N. Theobaldi

Those in which zygote formation seemed less abundant were :—

M. Rossii
M. Turkhudi
M. barbirostris

It should be noted, however, that in these experiments *M. Rossii* became infected, while in nature it has never been found infected by us.

There are, moreover, many considerations which lead to the conclusion that in nature all species of *Anopheles* are not equally concerned in the transmission of malaria.

We may have countless numbers of *M. Rossii*, as in Calcutta (environs), and get a malarial index of 0, and this appears to hold good in Madras, Bombay, and, as far as our observations go, universally. On the other hand, where we find *M. Listoni*, *M. culicifacies*, *P. Jeyporensis*, in India, we have a high endemic index.

The group of mosquitoes, those associated with intense malaria, are small dark mosquitoes with unbanded legs (*Myzomyia*, group 1).

M. Funestus and P. Costalis in Africa

The former mosquito is, like *M. Listoni*, which it closely resembles, a breeder in clean waters, streams, springs, etc., while *P. costalis* is found breeding in shallow pools about houses and frequents towns (in Africa), which *M. funestus* does not.

M. funestus was found by us to be infected in the Lagos hinterland to the extent of twenty-five to fifty per cent.

P. costalis, in Lagos itself, contained only three per cent. of sporozoits.

It is important then to determine precisely the species in a district and to determine the percentage of infection with sporozoits.

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Chapter XXIII

TO MAKE A MALARIAL SURVEY

Endemic Malaria

The clue to the epidemiology of malaria in the tropics is to be found in the infection of the native population of a country. The malaria of Europeans is merely the result of their exposure to infection from this source. Investigation into the natural history of malaria, therefore, resolves itself largely into the study of native or endemic malaria. It has always been recognized that in a particular country certain districts are more malarial than others. It was not, however, till Koch used the percentage of infected children as the test of the malarial intensity of a place that accurate measurement of this became possible.

To Investigate the Endemic Malaria of a District

(A) *The Breeding-Places of Anopheles*—

1. Examine all collections of water within half-a-mile. Stir up the mud of small puddles, and use a dipper where the water is weedy or difficult of access. Examine wells, 'chatties,' streams, and swamps, as well as pools of every description. Take specimens of larvae from each, placing in specimen tubes and labelling.

2. Determine the species of the larvae collected.

3. Make a map of the neighbourhood, noting—

(a) All breeding grounds.
(b) What species are found breeding in those examined (Fig. 60).

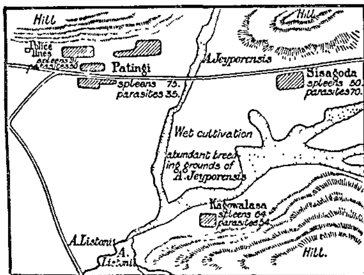


Fig. 60. Map shewing how to make a Malarial Survey

(B) *The Presence of Winged Anopheles*—

1. Search in outhouses, under eaves, etc., as described in Chap XIII, for *Anopheles*. Determine the species, note relative numbers of each species on map. The relation of *Anopheles* to native dwellings will probably be evident.

2. In the dry season the search for *Anopheles* may be negative, and there may be no breeding-places. Make in the most sheltered places small cement pools, and keep these filled with water. After a certain number of days they may contain young *Anopheles* larvae if the adults are present in the houses. (It is necessary to be sure one's water supply does not contain young larvae or eggs). The absence of the larvae in the pools does not necessarily mean, however, that adult *Anopheles* are not present in the houses (see choice of breeding-grounds by different species of *Anopheles*).

Note the result in the case of each test pool.

3. In the conditions just described observe the pools made by the first shower of rain of the on-coming 'rains.' Note after three days have passed the presence of larvae in many of these. Note the presence of these on the map. The distribution of *Anopheles* at the end of the DRY *season will usually be found to correspond to that* of native huts.

The Prevalence of Malaria

If we proceed to ascertain to what extent malaria prevails in a district we may attempt to do so in several ways.

1. We may consult hospital statistics and returns of death from malaria. This method is open to such grave error that it is extremely doubtful whether it is worth the labour bestowed upon it.

2. We may determine to what extent enlargement of the spleen occurs. This method has been largely used.

Precautions Necessary in Applying the Spleen Test

1. *The Age of the Individuals Examined.*—The enlargement of the spleen due to ordinary malarial infection tends to disappear once the individual has ceased to suffer from malarial infection. In very malarious countries, where each individual, after childhood, has become highly immune, the adult population usually shew no splenic enlargement (Tropical Africa).

In less malarious regions the adults have not become highly immunized, and a certain number of them will be found with enlarged spleens and malarial infection The use then of the percentage of adults with enlarged spleens is not a reliable method of determining the real intensity of malaria.

In children, the spleen enlargement appears to require a certain time to become apparent, and it takes a certain time to disappear, as the malarial infection disappears with ensuing immunity

In the examination of children for splenic enlargement and the presence of parasites in their blood, we found

(i) In the early ages, one to two years, the number infected is usually in excess of those shewing splenic enlargement.

(ii) *Above two years, the spleen rate is* usually somewhat in excess of the parasite rate

(iii) Above ten years, the spleen rate is usually considerably in excess of the parasite rate.

In the use of a spleen census one should then avoid a mixed adult and child count, and children

between two years and ten years of age should be chosen.

2. *The District in Question.*—It seems clear that the comparison of the malaria of widely different regions by means of the percentage of enlarged spleens in the children is not possible. We have, however, found that in Bengal, the parasite rate and the spleen rate in children varied proportionally, the spleen rate was, however, nearly always about double that of the parasite rate.

3. *Time of Year. Seasonal Variations.*—We may determine by actual blood examination how many individuals have parasites in the peripheral circulation. By the use of the parasite rate in children up to ten years of age we get a definite and true index of endemicity which may be used in the comparison of one locality with another.

4. To the last method we would add, as a complimentary one, the determination of the percentage of infected *Anopheles* as giving the actual risk of infection in a district.

The Determination of the Endemic Index of a Place

1. Place a number of cleaned slides in a slide box. Take a straight surgical needle, paper and pencil.

2. Choose any village or quarter of a town. Get the assistance of a native with local influence, the native magistrate in an Indian bustee, the chief in an African village. Instruct him to muster the children of the village. The free display of 'pice,' half-pence, etc., will greatly aid one,

and by palpating a few spleens previously to taking blood specimens the children will come readily. It is well first to take the blood of one or two adults or big boys so as to allay fears. In all cases it will be found best to take for granted the willingness of the child, and if the operation is quickly and quietly performed there is little objection, especially when each receives payment.

3. Make dry blood films by the method described in the early part of the book.

4. At the same time a spleen census may with advantage be made.

On examining the films determine :-

(i) Number shewing parasites or pigmented leucocytes in the blood.

(ii) The species of each parasite present and the percentage value for each if the numbers are large enough.

To Determine the Infection in the Anopheles

(The Sporozoit Rate)

1. *Collect as large a number of Anopheles as* convenient from the village in and around which the previous observations have been made.

2. Dissect as many specimens as possible, noting in each case the species dissected, and noting in which species, if any, sporozoits are found.

In many cases the sporozoit rate is extraordinarily low, *e.g.*, two per cent., although *Anopheles* are abundant and the malarial index

is not low. In others, especially in African bush stations, the percentage may reach fifty per cent.

3. Leave specimens not dissected for several days and examine the mid-gut for zygotes.

Malarial Infection of Europeans

Although malaria is an infectious disease, and can arise only from an original human source, yet in the tropics we can no longer consider the origin of infection as occasional and due to the presence of other cases of 'fever.' In the tropics, and especially in Africa, we are dealing with a disease which is a normal condition of childhood, and which, with the coincident infection of *Anopheles*, is the usual accompaniment of every native hut.

European malaria in the tropics is, indeed, chiefly dependent on two factors—

1. The degree of exposure to native malaria, *i.e.*, the proximity to native dwellings.
2. The endemic index of the native dwellings in question.

To Investigate European Malaria

1. Examine the blood of as many Europeans as possible. Enquire *carefully* whether the person is taking quinine at the time, also take the temperature.

(i) The number shewing parasites or crescents.

(ii) The presence of pigmented leucocytes.

(iii) The presence of an increase of the large mononuclear leucocytes.

In every case make a differential count of the leucocytes and keep the record.

Observe especially, any community of Europeans shewing a larger percentage than usual of malarial infection. Note the conditions under which these are living, and note also the probable greater prevalence of blackwater fever in these communities, *e.g.*, Roman Catholic Fathers, West African miners, railway communities, Europeans in poor circumstances living in the slums of native towns, etc., Syrian hawkers, etc. Note those *communities habitually taking quinine.*

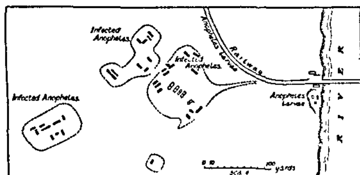


Fig. 61 Shews how Europeans are infected with Malaria from the native (children)

2. Note the usual relation between the degree of ill-health and the proximity of native huts. Make a map shewing European dwellings and shewing huts and hovels in relation with these (Fig. 61).

3. Make a thorough investigation of the conditions in these huts

(i) The percentage of infected children in each group.

(ii) The degree of infection of the adults.

(iii) Roughly estimate the number of *Anopheles* present, whether swarming, abundant, scanty, or impossible to detect by search. In the latter case make several 'test pools.'

(iv) Determine the species present and the relative numbers of each.

(v) Determine the sporozoit rate for each species.

(vi) Carefully map all breeding-places, noting what larvae are found.

4. Capture as many *Anopheles* as possible in the European houses, especially in the morning, and by looking within the nets. Determine the species, sporozoit rate, and from where probably derived. Examine the ovaries and spermatheca, and note whether freshly hatched or impregnated females are chiefly found. Note the presence or absence of males.

In investigating the malaria of any such settlement, native and European, continue the observations if possible throughout the year. Make observations on—

1. Seasonal variations in the endemic index (percentage of infected children).

2. Seasonal variations in the number of cases among Europeans.

3. Prevalence of any particular species of *Anopheles* at any time of the year.

4. Distance of flight of *Anopheles* from breeding-grounds, etc.

5. Sporozoit rate of *Anopheles* at different times of the year.

6. Examine especially the conditions where *Anopheles*, breeding-places, native huts, opportunity *for constant importation of malaria and numerous* susceptible children exist, and yet there is a complete absence of endemic malaria. In Africa it will probably be *impossible to find such places*, but they occur in India.

Endemic Areas of a Country

The map (p. 253) shews how the endemicity of large areas of a country is a very variable one. When opportunity offers, the endemic index should be determined for each locality, and, as far as possible, all the other facts detailed above. But the simple taking of the blood of a number of children (under ten) in any village gives at once valuable information as to malaria of the district, information which often is quite unsuspected. Thus, as is shewn in the map, the endemic index of Calcutta is 0, that is to say, in the immediate environs (not in the town itself) where practically the condition is one of a number of isolated villages, there is no malaria among the native children. At Jalpaiguri the figure is low, twelve per cent., but on reaching the foot of the Himalayas, we find the extremely high figure seventy-two per cent. In this case we were able among other differences to find a different species of *Anopheles*, which, as we have seen, is undoubtedly an important factor.

In other cases, however, all the conditions may be apparently identical, but within a distance of even ten miles we may get a change from an endemic index of 0 (Madras) to ninety (Ennur).

(i) The percentage of infected children in each group.

(ii) The degree of infection of the adults.

(iii) Roughly estimate the number of *Anopheles* present, whether swarming, abundant, scanty, or impossible to detect by search. In the latter case make several 'test pools.'

(iv) Determine the species present and the relative numbers of each.

(v) Determine the sporozoit rate for each species.

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In other cases, however, all the conditions may be apparently identical, but within a distance of even ten miles we may get a change from an endemic index of 0 (Madras) to ninety (Ennur).

These differences hold good in other countries, *e.g.*, in Italy. Here the mortality from malaria in the north is comparatively trifling, while in the south and the islands it is severe.

Here the difference may be due to differences in climate, but this explanation does not suffice in the examples in India we have mentioned.

Again, we have great irregularities in the distribution of the species of parasite. The quartan, for instance, in the Duars (Bengal) is exceedingly common amongst the native children, but in Lahore it is rare.

Similar differences have been noted in Algeria, where over large areas the quartan parasite is extremely rare, yet in a few localities it occurs in seventy per cent. of cases (BILLET).

So *in India, as a whole, we have certain* small areas where malaria is intense, *e.g.*, the Duars, Jeypore (Madras), and Kanara (Bombay) (CHRISTY), where we also find blackwater fever; yet in others, as in the Central Provinces, where apparently all the conditions are favourable, we have only a moderate intensity.

We require, then, to examine carefully the endemic indices over large areas in order to get an accurate idea of the variations in endemic malaria. The instances we have given will shew how erroneous it is to say *broadly*, '*such and* such a country is highly malarial,' for while this *may be true of one district it might be quite untrue* of another.

Further, after having established these broad data, it will be necessary to make a close survey of each individual district in order to endeavour to explain the factors at work.

Chapter XXIV

CLINICAL STUDY OF MALARIA

Enumeration of Red Cells

In blood counting, much practice only can give accurate results; inaccurate results are misleading and useless. For comparative purposes, counts should be made always at the same time, if possible, to obviate the effect of food, etc.

For diluting the blood, 0·9 per cent. salt solution may be used, or preferably, Toison's fluid, which has the following formula:

H_2O	160 c.c.
Glycerin .	30 c.c.
Sodium sulphate	8 grammes
Sodium chloride	1 gramme

Methyl violet (or other stain sufficient to colour the nuclei of the leucocytes)

The diluting fluid must always be poured into a watch glass, and it should not be sucked up out of the stock solution

No pressure must be used to make the blood drop exude from the finger.

Blood is then sucked up to the mark 1 on the pipette, and the end of the pipette carefully wiped before plunging it into the Toison's fluid The Toison is sucked up to the mark 101 exactly. The pipette is then rotated between finger and thumb,

so as to ensure thorough mixing. After rejecting the first few drops, which consist of diluting fluid simply, a small drop of the mixture is blown on to the counting chamber, and the coverglass applied as rapidly as possible. No fluid should escape into the moat or under the coverglass. When NEWTON'S rings are seen between the coverglass and side of the chamber, the former is in its right, closely-applied position.

In counting the corpuscles in each square, include, of those that touch or overlap the sides, *only those on the left hand and top side or right* hand and bottom side. Count at least 1,000 cells, the error is then only about two per cent.

The number of corpuscles per mm.³

$$= \frac{\text{No. of corpuscles counted} \times \text{dilution (100)} \times 4{,}000}{\text{Number of squares counted.}}$$

The normal average values are for man 5,000,000, for woman 4,500,000.

TOTAL LEUCOCYTE COUNT

The leucocytes may also be counted at the same time as the red cells, *i.e.*, from the pipette used for the red. This method has the advantage that all errors effect both counts equally, and the true ratio of red to white may still be got. Now for counting the leucocytes much larger fields are necessary than in the case of the red, as for every five hundred red cells there is only one white, so that counting chambers *especially ruled are often* sold for this purpose, but their use is unnecessary and may be obviated by the following method.

We require simply to determine the area of the whole microscope field with a given eye-piece, objective and given *length of tube*

1. One division of a Thoma Zeiss square = ·05 millimetre.

2. So that if the diameter happened to cover eight divisions the diameter would be ·4 millimetres, or the radius 2 millimetres.

3. The *area* of the field will therefore be πr^2, and the corresponding volume of blood $\pi r^2 \times \frac{1}{10}$ ($\frac{1}{10}$ = depth between coverglass and chamber, *i.e.*, $\pi r^2 \frac{1}{10} = \frac{1}{79{\cdot}51}$ cubic millimetres.

4. So that to get the number of leucocytes in the cubic millimetre we must multiply by 79·51, but it would be much more convenient to multiply by 100. In that case where $\pi r^2 \times \frac{1}{10} = \frac{1}{100}$, $r = {\cdot}1785$ millimetres, or diameter of field is ·357 millimetres. We require, therefore, to arrange our microscope so that the diameter is of this value, and this is readily done.

Two observations only are necessary.

1. Draw the tube of the microscope out so that diameter of field covers exactly eight divisions of the Thoma Zeiss chamber; note length of tube. Let this $= x$.

2. Draw tube out so that diameter covers exactly seven divisions. Note length of tube $= y$.

3. Therefore an increase in tube length of $y - x$ reduces diameter from eight divisions (·4 millimetre) to seven divisions (·35 millimetre); difference = ·05 millimetre.

4. *Required to calculate what increase in* length will cause reduction from ·4 to ·357 (difference = ·043).

$$\text{This will be } \frac{y - x}{{\cdot}05} \times {\cdot}043$$

This calculation is made, once for all, by the observer for his microscope, and the tube is drawn out the required amount, using, of course, the same eye-piece and objective.

To find total number of leucocytes per mm.[1]—

$$\frac{\text{Total number counted} \times 100 \times \text{dilution } (100)}{\text{Number of microscope fields counted}}$$

Count one hundred if possible.

The leucocytes may also be counted in the special pipette for white cells, but here again the method of counting by using the whole microscope field should be used. If the 'white' counter is used, the diluting fluid should be acetic acid 0·3 per cent. Sufficient gentian violet or methyl. violet is added to this to colour the nuclei.

To Clean Pipettes

For any accuracy of observation the pipettes should be scrupulously clean. There should not be the slightest tendency for the glass ball to stick to the sides. After a count has been made, the rubber tube is removed and the contents ejected by blowing from the pointed end.

1. Suck up dilute acetic acid so that all traces of stain are removed.
2. Suck up several lots of clean water to remove the acid.
3. Then absolute alcohol two to three times to remove the water.
4. Then ether two to three times to remove the alcohol.
5. Finally, blow hot air through with a syringe, the glass barrel of which may be heated in a flame (or simply *suck* air through).

These procedures take a very short time, and it is a satisfaction to know that the pipette has been put away perfectly clean and ready for the next observation.

The Estimation of the Haemoglobin

Gower's haemoglobinometer is the simplest and best. In sucking up the blood take care not to hold the tube too vertical, as the blood readily flows out from the rather large calibre of the tube. Order from a good maker, as several inferior instruments are on the market. The round form of tube is more easy to manipulate than the flat.

The standard of comparison in this apparatus is pikro-carmine gelatine, the colour of which corresponds to a one per cent. watery solution of normal blood.

All blood counting apparatus, etc., can be got from T. Hawksley, 357 Oxford Street, London, W.

Dare's *haemoglobinometer is accurate.* It possesses the advantage of dispensing with a pipette. It costs £4

To Count Platelets

Diluting fluid · glycerine saturated with *dahlia, and two per cent. saline solution,* take equal parts of these.

The ratio of platelets to red cells is 1:8 about. The absolute value per mm.³ 635,000 about.

Differential Counting of Leucocytes (*vide* page 41).

The Leucocytes in Malaria

We shall consider (1) the total leucocytes, (2) the percentage value of each kind.

The Total Leucocytes.—We may take 10,000 as the normal value *per mm.*³, and as 5,000,000

is the normal value for red cells, the proportion of white to red is

$$\frac{WC}{RC}=\frac{10,000}{5,000,000}=\frac{1}{500}$$

Now, in malaria, we may find two conditions, either that the total number of leucocytes is considerably below the normal value 10,000, *i.e.*, there is leucopenia or hypoleucocytosis, or that the total number is much above 10,000, *i.e.*, leucocytosis. If there is leucopenia, say, for instance, the total number is 5,000 instead of 10,000, then $\frac{WC}{RC}=\frac{5,000}{5,000,000}=\frac{1}{1,000}$, *i.e.*, the fraction $\frac{WC}{RC}$ is smaller than normal.

If, on the contrary, the total leucocytes are 20,000 instead of 10,000, *i.e.*, leucocytosis, then $\frac{WC}{RC}=\frac{20,000}{5,000,000}=\frac{1}{250}$, *i.e.*, the fraction $\frac{WC}{RC}$ is greater than normal.

It is this ratio $\frac{WC}{RC}$ that it is important to determine, for unless the red cells are counted as well as the white, little value attaches to the leucocytic value.

Turning now to malaria, we find that we get changes of the following kinds:—

(1) 11 a.m., rigor. Red cells = 2,900,000. $\frac{WC}{RC}=\frac{1}{290}$,
White cells 10,000 - -
i.e., leucocytosis.

(2) 11.30 a.m., rigor completed - $\frac{WC}{RC}=\frac{1}{764}$,
i.e., leucopenia.

(3) 2 p.m., temperature 38·2° - $\frac{WC}{RC}=\frac{1}{968}$,
i.e., increased leucopenia.

The leucocytosis was, in this case, quite transient, followed by a marked leucopenia.

During the course of an attack, we may have changes of this kind :—

1. Some days before the attack and before parasites appear in the blood, instead of $\frac{WC}{RC}=\frac{1}{500}$ $\frac{WC}{RC}=\frac{1}{1,000}$ *i.e.*, a leucopenia.

2. During the shivering attack and height of the pyrexia, the condition changes to one of leucocytosis, so that

$$\frac{WC}{RC}=\frac{1}{300},\ \frac{1}{200},\ \text{or even}\ \frac{1}{90}$$

3. This leucocytosis may not last long, but is followed again by a marked *leucopenia* which is at its maximum before the onset of the next attack.

$$\frac{WC}{RC}\ \text{instead of}\ \frac{1}{500}\ \text{may be}\ \frac{1}{800}$$

BILLET (Fig. 62), who has traced out hourly the relation of the leucocyte curve to the temperature curve, has shewn that in regular curves of the tertian or quartan type, the leucocytic curve follows closely the variations in the temperature. Thus, before the febrile attack in a quartan, there may be a leucopenia represented by $\frac{WC}{RC}=\frac{1}{1200}$ at the time of the attack, however, there is a leucocytosis of $\frac{WC}{RC}=\frac{1}{200}$.

This gradually disappears, passing through the normal value $\frac{1}{500}$, and again reaching a marked *leucopenia* before the next attack The variations are of the same kind in irregular

temperatures, the leucocytosis corresponding to the rise of temperature, and the leucopenia to the apyretic intervals.

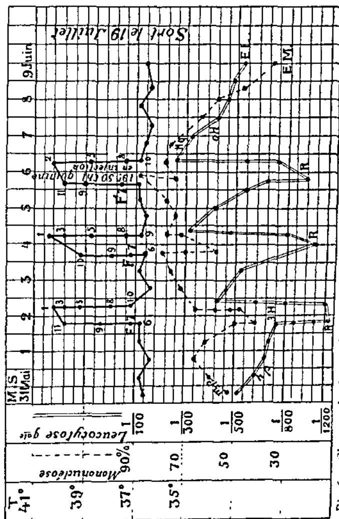


Fig. 62. Shewing the changes in the total Leucocytes (and in the percentage of mononuclears large and small) in a case of simple tertian fever. The double line curve = that of total Leucocytes. (After BILLET)

The Percentage Value of the Leucocytes.—If we now make a differential count in a stained specimen we shall be able to ascertain what change, if any, there is in the relative percentage of the different kinds (for normal values *vide* p. 42).

1. The main *characteristic* change is that there is an increase in the percentage of large mononuclears, so that at times they may even outnumber the polynuclear

2. The change is especially well-marked in the periods of apyrexia (*i.e.*, when there is a leucopenia). When there is a leucocytosis the increase in the mononuclears may not be apparent

As examples of this leucocytic change, we may give the following —

(i)	Small mononuclear	18·1 per cent.
	Large mononuclear and transitional - -	31·4 ,,
	Polynuclear - -	50·2 ,,
	Eosinophil	0·4 ,,

A fatal case of malignant tertian (BASTIANELLI).

(ii)	Small mononuclear -	19·1 per cent
	Large mononuclear and transitional - -	41·0 ,,
	Polynuclear - -	39·0 ,,
	Eosinophil -	0·6 ,,

A fatal case of comatose malignant tertian (BASTIANELLI).

* PANSE.—Malignant tertian fever, t 37·2° C

(iii)	Small mononuclear -	18·1 per cent.
	Large mononuclear and transitional -	26·4 ,,
	Polynuclear -	55·3 ,,

* *Zeitschrift fur Hygiene*, 1903 p 592

t. 97·6° F. Malignant tertian :

(iv)	Small mononuclear	-	14·8 per cent.
	Large mononuclear and transitional -	-	46·7 „
	Polynuclear -	-	38·5 „

The figures are by no means always as high as this, but, as we have already said (p. 41), we consider a value above fifteen per cent. as diagnostic of malaria. The higher values are appreciated at once by an inspection of the slide where the large mononuclears seem to occur in every field, and may be pigmented. For the low values a careful count is required.

An increase in the large mononuclears has been found in one case of human trypanosomiasis. This has not, so far, been confirmed, but if it is, it can but slightly effect the value of the counts in malaria as a diagnostic means for the clinical features of trypanosomiasis, so far as known, are extremely characteristic, and the chance of an *European* being infected with the disease does not appear to be great, the two known cases having occurred in tropical Africa. Further, together with the increase of the mononuclears in malaria there are, if thorough search is made, also pigmented leucocytes to be found. The *relative* count of malaria is of great assistance in at least two conditions, (1) in those cases where quinine has been taken, (2) where consequently the diagnosis is uncertain and the question of typhoid fever arises. As we shall now see, the *relative* count in typhoid is quite different from that of malaria.

Typhoid Fever

During the first week (of uncomplicated cases) the leucocytes are normal.

During the second week there is a *leucopenia*, *e.g.*, 2,000, and the leucopenia is in proportion to the severity of the disease.

During the third and fourth weeks the *leucopenia* is still more marked, though also a leucocytosis may be found without any apparent cause.

Relative Leucocyte Values.—During the third, fourth, and fifth weeks the mononuclears, *large and small*, may reach the values of forty to sixty per cent., and among these the proportion of *small* mononuclears is very striking.

Pneumonia

There is very early a leucocytosis, *e.g.*, 25,000, four hours after the initial chill. The maximum occurs, as a rule, just before the crisis. The number may fall from a high value to normal in twenty-four hours. Leucocytosis is said to bear a relation to the amount of exudation (*i.e.*, lobes involved).

Relative count—

Large and small mononuclear	2 to 4 per cent.
Polynuclear	90 to 95 ,,
Eosinophil	rare.

The Widal Reaction in Typhoid

While we consider, it is not going too far to say that typhoid and malaria can be readily distinguished by the leucocytic count, yet seeing that

in the WIDAL reaction we have an easy means of diagnosing typhoid, the application of this test is of the greatest service in those cases where the diagnosis of malaria or typhoid remains doubtful. We shall describe briefly how the test is carried out. There is no necessity for specially constructed bulbs or graduated pipettes, as often thought.

1. Draw out a piece of glass tubing, so as to make a pipette, having a fine end about the diameter of a hypodermic needle.

2. Collect enough blood to fill the pipette to the height of about half-an-inch. The blood will readily flow in if the pipette is held sloping downwards. Seal off the fine end in a flame. Centrifugalize, if convenient, but abundance of serum can be got without by allowing to clot.

3. *To Dilute the Serum.*—Draw out a piece of glass tubing into a long fine filament; take a piece about six inches long; make an ink mark about half-an-inch from the end of the tube; insert *this marked end into the tube containing* serum (and clot) and allow serum to flow up to the ink mark; then let a distinct bubble of air follow (the size of this bubble does not signify); next allow broth to flow up to ink mark; repeat this procedure until nine drops of broth are in the tube; these are now each separated by an air bubble, and also by a bubble from the serum. The dilution is now one in ten.

Blow out all the drops on to a slide or watch glass, and mix by sucking up and blowing out a few times.

4. Take a drop of the diluted serum in a fresh piece of tubing, make a mark as before, and then allow broth to flow up; this gives a dilution

of one in twenty, a second drop one in thirty, third drop one in forty, and finally a drop of typhoid emulsion; this gives a dilution of serum of one in fifty, containing typhoid bacilli. The whole process of dilution takes less than five minutes.

5. *Typhoid Emulsion.*—A bacillus should be used that is known to be active. Take a fresh over-night agar culture and make a fairly thick emulsion in broth or salt solution.

6. *Dilution and Time Reaction.*—A dilution of one in fifty with a time limit of half-an-hour may be used. With a less diluted serum the time limit must be less.

7. Whatever time limit and dilution be used, it is very necessary to perform controls from time to time with a variety of other cases to make sure that the agglutination, if produced, is not produced by normal sera.

The Isotonic Point or Tonicity of the Blood

If a drop of blood is allowed to drop into a one per cent. solution of salt in a small test tube and stirred up, the uniformly turbid solution will eventually become clear when the corpuscles have settled at the bottom and the supernatant fluid will be unchanged; if, on the contrary, we add another drop of blood to a little water in a test tube the whole drop is immediately *laked*, and we have resulting a solution of haemoglobin. The former solution of salt is called hypertonic, the latter solution of water hypotonic. Now, if we start with such a hypertonic solution, one per cent. salt, and proceed gradually to dilute it, we shall

eventually reach a strength where the hypotonic, *i.e.*, haemolysing effect begins to appear. The strength of salt solution just above this where no change occurs is the isotonic point for the particular blood in question. This point then gives us information as to the resistance to a haemolytic action of the corpuscles. The blood in various diseases is found to vary in regard to the strength of salt required to prevent haemolysis. So that if a normal blood is unchanged by a 0·5 per cent. salt solution, whereas an abnormal requires 0·6 per cent. to protect it, the latter blood is described as having a *less* resistance than the former, but it has a *higher* isotonic point.

The determination of the isotonic point then gives us a more definite notion of the state of the blood in disease than does a mere determination of the haemoglobin. The isotonic point of human blood is about 0·41 per cent. salt solution.

To Determine the Isotonic Point

1. Measure out one c.c. of each salt solution of descending strengths, 0·43 per cent., 0·41 per cent., 0·39 per cent., etc., into four small test tubes and one c.c. of water into a fifth tube.

2. Add to each the amount of blood contained in two divisions of the stem of a Thoma-Zeiss pipette (the whole stem contains ten divisions).

3. Allow to stand for some time. Some of the solutions will have haemoglobin in solution.

4. The amount of haemoglobin in each tube can be estimated by adding the amount of a normal blood in two divisions to one c.c. of water. Call

this = 100 per cent. Dilute this solution, so that *a number of tubes equal to ninety, eighty, seventy,* etc., per cent. are got. Compare the tubes containing the salt solutions directly with these.

In malaria, the resistance of the blood is markedly lowered, thus, whereas in a control normal blood a 0·41 per cent. salt solution gave no haemolysis; in the case of two malaria patients, the haemolysis was equal to twenty-five per cent. and forty per cent., respectively.

In blackwater fever, on the contrary, a raised resistance of the blood may be found.

Clinical Study of Malaria

The Urine.—While not proposing here to consider the general reactions of the urine in malaria, for which we must refer the reader to any standard text-book, yet we think it useful to consider some points which are of more particular interest. It is especially in blackwater that we still require complete analyses of the urine, and more especially in those who are constantly subject to malarial attacks and are at the same time taking quinine. It is possible that such analyses might give us indications which would enable us to avert the danger of an attack of blackwater fever and to determine when quinine should not be given. We have not considered here the method of examining the urine by 'cryoscopy,' as it is not at present a practical clinical method, but its possibilities should not be forgotten

Albuminuria.—The occurrence of albuminuria in malaria varies according to the particular country; thus in Rome it is uncommon, in Senegal,

on the contrary, exceedingly common. This is an *illustration of the often neglected fact that tropical* malaria differs in many ways from malaria of temperate climes.

Filter the urine if morphological constituents are present, as is the case in blackwater fever, through two thicknesses of filter paper, or add some calcined magnesia, then filter. Place some urine in a urine glass and, with a pipette reaching to the bottom, allow half as much nitric acid to slowly trickle in (SIMON). A white cloud at the junction layer indicates *serum albumin* (globulin or peptones). Urea nitrate crystals will often separate out at this junction layer.

Serum Globulin.—Make the urine *alkaline* with ammonia; filter off any precipitated phosphates; to the urine add an equal volume of saturated solution of ammonia sulphate. A precipitate indicates globulins; or the formation of the precipitate may be seen at the junction layer. Test filtrate for albumin by adding excess of acetic acid and boiling.

Albumoses.—Acidify the urine with acetic acid; add an equal volume of a saturated solution of salt; boil; if a precipitate occurs (albumen) filter hot. Albumoses separate out on cooling; or to the hot filtrate add caustic soda solution, then dilute copper *solution gradually*; a *red colour* signifies albumoses.

NOTE.—Urines rich in urobilin (*e.g.*, malaria and blackwater fever) will give this biuret reaction.

In presence of urobilin: to ten c.c. of urine add eight grammes of powdered ammonium sulphate until dissolved; boil for a few seconds; the albumoses are precipitated on the sides of the

test tube; pour off the urine, and wash the precipitate with alcohol, then chloroform; dissolve in water and apply the biuret test. Test the alcoholic extract for urobilin.

Nucleo-Albumens.—Filter the urine carefully; boil to remove albumen; then add gradually excess of strong acetic acid. A turbidity indicates nucleo-albumens.

BLOOD (HAEMOGLOBIN, ETC.)

1. *Examine Spectroscopically* (Fig. 63).—If the bands of methaemoglobin or oxyhaemoglobin are seen, confirm by adding ammonium sulphide when the bands of reduced haemoglobin are got.

2. *Heller's Test.*—Make the urine strongly alkaline with caustic soda; boil; the precipitate in the presence of haemoglobin is bright red; confirm by dissolving the filtered precipitate in acetic acid, a red solution is formed (spectroscopically this gives the characteristic bands of haemachromogen).

3. *Guaiacum Test.*—Equal parts of tincture of guaiacum and oil of turpentine (which has been exposed to the air) are taken; add slowly to the urine. A blue ring is formed at the junction layer.

METHAEMOGLOBIN

The urine in blackwater fever when examined early, most frequently contains blood pigment in this form, later oxyhaemoglobin. This, according to HOPPE-SEYLER, also holds good for every urine with haemoglobin in solution.

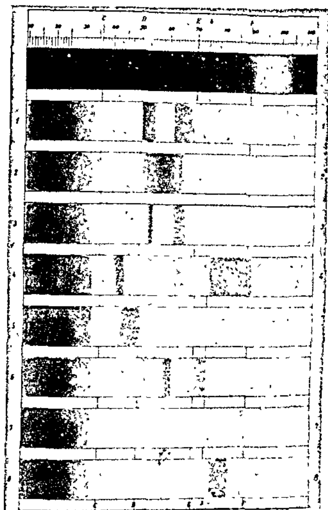


Fig. 63 Spectra of (1) *Oxyhaemoglobin*; (2) *Haemoglobin*; (3) *Alkaline Methaemoglobin* (4) *Methaemoglobin in Neutral or Acid Solution*. (5) *Alkaline Haematin*. (6) *Reduced Haematin Haemochromogen*. (7) *Urobilin*. (8) *Zinc-Urobilin*

The Characters of Methaemoglobin are:—

In *acid* solution the oxyhaemoglobin bands are weak or invisible. There is a band *between* C and D, nearer the former. The band of acid haematin is similar in position. It is, however, close to C.

In *alkaline* solution the acid band disappears, and a faint band on the red side of D takes its place (compare with alkaline haematin).

Reduced by ammonium sulphide, the bands of reduced haemoglobin are got. It differs from oxyhaemoglobin in its chemical reactions by the fact that it is precipitated by basic or neutral lead acetate solution, whereas oxyhaemoglobin is not

Detection:—

1. In presence of oxyhaemoglobin. Ppt. with basic lead acetate; filter, decompose the precipitate with carbonate of soda solution; examine for the bands of alk-methaemoglobin.

2. In presence of urobilin. Proceed in the same way.

3. In presence of bile pigment. Precipitate these by making the solution alkaline with ammonia after adding $CaCl_2$.

4. In neutral solutions its spectrum is identical with that of haematin in natural solutions (NEUBAUER and VOGEL). Reduced by $(NH_4)_2S$, methaemoglobin is changed to reduced haemoglobin and haematin to reduced haematin, the bands of which are easily recognized.

UROBILIN

Frequently occurs in the urine in jaundice instead of bile pigment.

According to HAYEM, it is associated with methaemoglobinaemia. Its occurrence in blackwater fever is very common, occasionally before the attacks, but more constantly after the oxyhaemoglobin has disappeared or together with it.

Characteristics :—

1. In *acid* urine a band near F occurs, between 88 and 101.

2. In *alkaline* urine a band between 81 and 95.

3. Make the urine strongly alkaline with ammonia filter, add $ZnCl_2$ solution, but not sufficient to form a permanent precipitate.

A green fluorescence occurs, and the much clearer band, nearer 'b' than the acid band.

Detection :—

1. If oxyhaemoglobin is present. Precipitate the urobilin with *basic* lead acetate, then acidify the precipitate, when the urobilin goes into solution.

2. If methaemoglobin is present. *Neutralize* the urine with carbonate of soda; precipitate the methaemoglobin with neutral lead acetate. Filter; test the filtrate for urobilin.

BILE PIGMENTS

Where urobilin is present, as in blackwater, the colour of the foam on shaking the urine, the staining of the filter paper, etc., cannot be regarded as satisfactory texts.

Detection :—

1. *Gmelin-Rosenbach Test.*—Filter the urine through filter paper (Swedish). Dry; apply a drop of nitric acid (fuming) to this, a play of colours is got.

2. *Huppert's Test.*—Precipitate the urine with $BaCl_2$. Filter; wash the residue off the filter (perforated) with acidulated H_2SO_4 alcohol. Boil. A bright green colour indicates bilirubin.

3. *Smith's Test.*—To ten c.c. of the urine add two c.c. of dilute tincture of iodine (tincture of iodine 1, alcohol 10). A green ring forms at the junction zone.

BILIRUBIN AND HAEMATOIDIN (IN URINARY SEDIMENT)

1. Bilirubin crystals form yellowish-brown rhomboidal plates or needles

Easily soluble in $CHCl_3$. Gives GMELIN'S reaction, green, under the microscope.

2. Haematoidin, dark-red in colour or greenish if impure, with nitric acid they give a transient blue.

According to HOPPE-SEYLER, however, they are identical.

HAEMATOPORPHYRIN

Occurs in urine as alkaline haematoporphyrin (Fig. 64). In urate sediments a similar form occurs. It is soluble in chloroform, giving bands similar to those of oxyhaemoglobin, but acid converts this into acid haematoporphyrin bands. Solutions have a brilliant red fluorescence. It is found in the urine in toxic conditions, such as chronic sulphonal poisoning. It is precipitated by lead acetate, while oxyhaemoglobin is not.

SUGAR

Before testing for sugar, boil to remove all proteids.

Reduction of copper solution is effected by bile pigments. Reduction occurs also in patients taking salicylic acid, sulphonal, and quinine (SIMON), so that it may be necessary to use—

1. *Fermentation Test* or

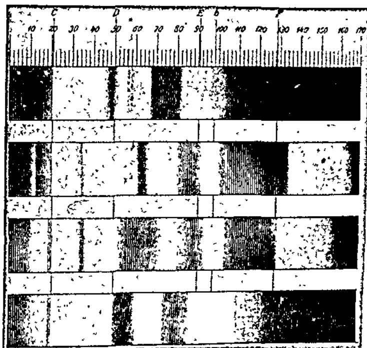


Fig 64 Spectra of (1) *Haematoporphyrin (in Acid Solution)*; (2) *Haematoporphyrin in Alkaline Solution*; (3) *in Neutral Solution*. (4) *Haematoporphyrin (urate-sediments)*

2. *Phenyl-Hydrazine Test.*—Take five drops of pure phenyl-hydrazine, ten drops glacial acetic acid, one c.c. of a saturated solution of common

salt; add three c.c. of urine; boil for three minutes; cool; crystals separate out in a few minutes up to one hour. *This is an exceedingly delicate test.*

The Detection of Quinine in the Urine *

The detection of quinine in the urine is of importance in connexion with the property that this drug has of inducing attacks of haemoglobinuria (blackwater fever) in patients resident in *regions where malaria is especially virulent*, and where generally the parasite form is the malignant tertian associated with an extremely high endemic index of native (children) malaria.

Two hundred c.c. of urine are acidified with some drops of sulphuric acid. A spoonful of solid picric acid is then added. The solution is allowed to stand for an hour and then filtered. The solution should be quite clear and should give with *a saturated solution of picric acid no turbidity*. If there is difficulty in getting a clear filtrate add a trace of egg albumen and filter again. The half-dry residue is then digested in an Erlenmeyer flask with fifty c.c. of 3·0 per cent. soda solution for half-an-hour on the water bath. Now add sixty c.c. chloroform; shake for two hours in a shaking apparatus. The solution of chloroform is now removed by means of a separating funnel and collected in a weighed flask. The flask should have a long neck to prevent spurting. Evaporate in a water bath and dry at 120° C. The residue is quinine. The experimental error is only one to two per cent.

* Kleine, *Zeitschrift fur Hygiene* Bd. xxxviii, H 3, s. 460

Determination of the Periodicity of Parasite Development

The inspection of a temperature chart is not in itself sufficient to determine the cycle of development of a parasite. Thus, as is well known, a quotidian temperature chart may be produced by a double tertian (simple) or by a triple quartan infection. If then, in the case of the double tertian, we made microscopical examinations at definite intervals for forty-eight hours, we should find in the blood at any particular time parasites in *two* phases of development corresponding to each cycle. The accompanying chart shews how, in the case of what proved to be the malignant tertian parasite, we were able to establish the cycle of development. We proceeded to make blood examinations at frequent intervals (four hours). We found that at any particular time parasites of various sizes might be found, but by counting several hundred parasites in each film and estimating their size with a micrometer we found that at any particular time there was a preponderance of parasites of one size. Thus, at ten p.m. on the 2nd, there are numerous small forms, *i.e.*, about one-seventh to one-eighth of a red cell in diameter, and it is not till ten p.m. (about) on the 4th that the same condition of blood is found again, accordingly the parasite had a developmental cycle of forty-eight hours (approximately). And, further, we determined the periods taken to develop from small forms to largest forms in the peripheral blood (about eighteen hours) and the disappearance of these and the reappearance of numerous youngest parasites (about thirty hours).

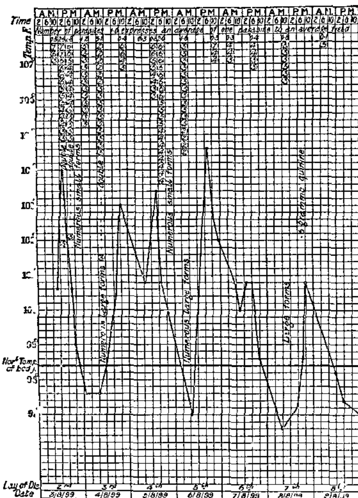


Fig. 65. Illustrating method of determing the developmental Cycle of a Parasite (The figures within the circles represent the size of the Parasites—thus 7 signifies the Parasite is one-seventh the diameter of a red cell)

So that by determing in these three periods we were able to conclude that the parasite was the malignant tertian.

In order then to determine the cycle of a parasite it is necessary:—

1. To estimate the size and percentage of parasites of each size at any particular time, *e.g.*, starting with the onset of the attack.

2. To follow each group to its period of maximum development in the circulation.

3. To estimate the time between this period and the next appearance of young forms.

4. To estimate the time between the appearance of an outburst of young forms (No. 1) and a second similar outburst (No. 4).

The interval between one and four should be equal to the sum of the intervals of periods two and three. It is more accurate to use a micrometer scale for measuring, but the estimation can be made with considerable accuracy without.

If we are dealing with three generations of parasites as in a triple quartan the principle is precisely the same, though it may require careful observation to separate the different groups, though in this particular case the process is facilitated by the presence of segmenting and pre-segmenting bodies which are easily counted. In order then to establish a parasite cycle, repeated observations at definite intervals are necessary, and also the temperature should be carefully recorded every four hours or two hours as considerable variations may otherwise escape observation.

Simple Tertian

Examined during the commencement of apyrexia, young parasites are found one-fifth to one-ninth the size of the red cell. The corpuscles *may be slightly larger than normal.* On the following day the parasites occupy one-half to two-thirds of the corpuscle, much pigmented, but not so actively motile as the smaller ones. The red cells are much enlarged at the end of the second day; presegmenting forms are found. Division into four to six or more parts may take place six hours before an attack, but true fission forms are only found two to three hours before an attack. These forms have sixteen to twenty spores; so that, here again, the multiplication of a group of parasites may be shown, microscopically, to coincide with a febrile attack. But, as opposed to quartan, the actual number of fission forms in the peripheral circulation is small; they are far more numerous in the spleen.

Double Tertian

The interval between the development of the two groups of parasites is about twenty-four hours. So that if a blood examination be made just before an attack, fission forms will be found, and parasites about half-grown. Here again, by following out the development periodically, these latter forms will be found to sporulate on the next day.

Quartan Parasite

If the blood be examined as soon as apyrexia sets in, the corpuscles will contain young parasites

one-fifth to one-sixth as large as the red cells; growth is progressive during the *apyrexia, and* six to ten hours before the next attack 'presegmenting' forms will be found. The parasite now fills the cell which is not enlarged. The *pigment* is arranged in radiating bands. The next stage is the concentration of the pigment in a central *mass, and it is seen in fresh or stained specimens* that the cytoplasm is divided into eight to ten segments or oval bodies (daisy form). This *segmentation is nearly coincident with the next* attack, but parasites in complete fission may be found five to six hours earlier. As the *temperature rises these sporulating forms disappear and,* again, young forms are found in the red cells. The quartan then goes through all its stages in the *peripheral circulation.*

Double Quartan

Two groups of parasites going through the above regular cycle, with about a day's interval, may be followed in the blood.

Triple Quartan

There are three distinct groups sporulating on successive days.

Malignant Tertian (Tropical)

During the pyrexia, small forms, one-eighth [one-fifth] of the red cell, may be found. *They persist during the pyrexia, i.e.,* for the greater part of a day. The parasites at this stage may be extraordinarily few.

During the apyrexia, forms one-fourth to one-third of a red cell are found, and the parasites are found in greatest numbers. Fission and presegmenting forms are extremely rare in the tropics. During the next attack the young forms are again found, and so the time of the cycle, as we have shown above, may be deduced, and may be controlled by observation of intermediate stages.

Quotidian

Parasites have been described which complete their development in twenty four hours (about). Thus, at the pyrexia young forms occur. During the apyretic interval large forms and presegmenting forms, and, again, at the next attack young forms, thus developing in twenty-four hours. As we have stated above, to establish accurately this cycle three periods would have to be traced —

No. 1 (? Twelve hours) from young forms to largest forms.

No. 2. (? Twelve hours) from largest forms to young forms

No. 3. Twenty four hours from young forms to young forms

While some consider that the quotidian temperature is due to the fact that the malignant tertian has a very variable period of development, viz., twenty-four to forty-eight hours, and, in fact, all intermediate times, others consider that with one generation of parasites there is a second accumulation of young forms in sufficient quantity to produce a quotidian attack

In quotidian fever, due to the malignant tertian parasite, the characteristic febrile attack,

with its preliminary pseudo-crisis, is lost. The attack instead of lasting about a day lasts a few hours only, as in the *simple tertian, and instead* of a pseudo-crisis there is a true crisis, but the young parasites, as in the malignant tertian attack, are still coming into the circulation, and there follows a rise which replaces the apyretic day in the ordinary malignant tertian.

According to MAURER, in the case of the quotidian chart produced *by one generation of malignant tertian parasites* we have the febrile attack produced by the division of the majority of the segmenting forms, and then a fall to normal occurs; when, however, there is a *sufficient* accumulation of young forms arising from the same generation there is again a rise giving the quotidian chart. As we have seen, the young forms of the malignant tertian parasite persist during the pyrexia. If, however, by any means they are destroyed or cease appearing temporarily during the day of pyrexia, we should get a fall to normal, and then as soon as this inhibiting cause was removed, again a rise, giving a quotidian chart produced by the malignant tertian parasite.

IRREGULAR TEMPERATURES

Besides the typical malignant tertian temperature chart and the quotidian chart, various irregular temperatures may occur, due solely to the malignant tertian parasite. Such charts are not at all uncommon in first attacks in the tropics, and may be followed by charts with regular curves.

The malignant tertian parasite has a developmental cycle of about forty-eight hours, and it seems

more likely that these irregular charts are produced by an irregular irruption of young forms into the circulation than that the parasite has a variable time of development. If we suppose that young fission forms exist in the internal organs, but do not commence their growth in the circulating red cells, but come into the circulation irregularly, then we should have still a constant time of development, but an inconstant time at which the development started. If, however, a quotidian parasite exists, there should be no difficulty, as we have stated above, in determining the fact by a series of measurements at fixed intervals

Action of Quinine

The data of different investigators into the absorption and elimination of quinine exhibit considerable differences dependent upon the different conditions of experiment and the mode of estimation employed. The following statements must therefore be received with caution:—

1. *According to* KERNER *the elimination of*—

Quinine hydrochlorate begins in fifteen minutes and ends in forty-eight hours.

Sulphate (neutral) begins in thirty minutes and ends in forty-eight hours.

Sulphate (basic) begins in forty-five minutes and ends in sixty hours.

2. *Mode of Administration*—

Per os, quinine appears in the urine in thirty to fifty minutes.

Per rectum, quinine appears in the urine in eighteen to twenty minutes

Subcutaneously, quinine appears in the urine in twelve to twenty minutes.

3. *Duration of Elimination*—

According to GAROFALO it lasts one-and-a-half to seven and three-quarter hours.

According to DIETL it lasts forty-eight hours.

According to BYASSON it lasts seventy-two hours.

According to PERSONNE it lasts eight days.

4. *The Acme of Elimination*—

According to THAU and KERNER after the first six hours.

According to GAROFALO after the first one-and-a-half to four hours.

According to KLEINE after the first three to six hours.

5. *Hypodermic Injection.*—GAROFALO states that the elimination is rapid, and that larger doses can be accumulated in the blood in a shorter time by this method than by doses given by the mouth, while KLEINE states that the absorption by this method is slow. KLEINE's figures will be given below.

6. *Amount of Quinine Eliminated*—

WELITSCHOWSKI	- 100	per cent. about.
KERNER	- 95	„
BYASSON	- 75	„
KLEINE	- 9·27	„ (*vide* later)
PERSONNE	- 16	„
MERKEL	- 13	„
MARIANI, during first day	- 18·7	per cent.
„ second „	- 6·3	„
„ third „	- 1·3	„
„ fourth „	- 0·7	„
Total	- 27	per cent.

7. KLEINE's data as to the amount eliminated in twenty-four hours:—

Per os Administration—

(i)	25·34 per cent.	
(ii)	19·71	,,
(iii)	27·29	,,
(iv)	9·67	,,

This low value, No. 4, is explained by the fact that the quinine was given on a full stomach, whereas in the three other results the quinine had been given to the patient fasting.

Per Clysma—	(i)	17·66 per cent.	
	(ii)	17·15	,,
	(iii)	17·84	,,
Subcutaneously—	(i)	11·37	,,
	(ii)	9·70	,,
	(iii)	15·32	,,

Now, although proportionately a smaller amount is excreted in this way (and this is possibly in conformity with the clinical experience that ringing in the ears and other unpleasant symptoms of quinine are generally absent after subcutaneous injection), yet it is probable that the excretion is a more prolonged one than by the other methods, for deposits of quinine can still be found at the site of injection some weeks later, and so the undoubted efficacy of this mode of treament may really be due to its prolonged action (and elimination).

MARIANI's results also shew that after an injection of quinine into the muscles of a rabbit, about twenty-four hours later, half the amount could still be extracted from the muscles. KLEINE and MARIANI's results shew that a full stomach inhibits markedly the absorption of quinine, so also any catarrhal state is prejudicial.

two to three frequently, five to six or possibly twenty-four hours.

2. The amount of quinine does not determine whether the haemoglobinuria is slight or severe.

3. After haemoglobinuria has been produced by quinine, a second administration does not necessarily produce a second attack of haemoglobinuria.

These facts clearly shew that it is not the *quinine, per se, but a condition of blood in the* particular malarial patient which is the determining factor whether quinine will produce an attack.

This is further borne out by the well-known fact that the aborigines rarely, if ever, suffer from haemoglobinuria, but it is in Europeans subjected *to unnatural climatic conditions and subjected to* virulent malaria that the disease is most frequently found.

We would only add, finally, that it is quite illogical to abstain from quinine in malaria, on the contrary, its *adequate* administration would prevent the occurrence of these attacks.

As we have *already said, an accurate study* of the urine in these cases and in allied cases of malaria where quinine produces urobilinuria is necessary.

Especially important is the study of the urine and the blood in the prehaemoglobinuric state. It would, of course, involve an accurate study of *all possible subjects of the disease, and* more especially those who had already had an attack.

Post-Mortem Changes in Malaria (Marchiafava and Bignami)

Brain:—

1. Punctiform haemorrhages of the meninges.

2. Punctiform haemorrhages of the white substance of the brain.

3. The brain capillaries may contain nearly every red cell infected. Sporulating forms are especially common.

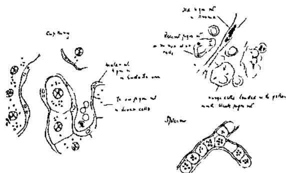


Fig 66, Shewing deposition of Pigment in Liver (left), Spleen (right), and Sporulating Parasites in Brain Capillary (bottom)

4. The capillary endothelium may show fatty degeneration, together with pigmentation, and sometimes parasites.

5. Similar appearances are also found in the vessel of the pia mater.

Lungs:—

1. Large pigmented mononuclears in the capillaries, but especially in the veins; in the lungs especially phagocytosis is proceeding.

2. There is a terminal infection with the diplococcus *pneumoniae*.

Spleen.—The trabeculae of the pulp are distended by infected red cells, and pigmented large mononuclears are abundant. The malpighian follicles, on the contrary, are non-pigmented.

Liver. -Endothelium of capillaries is swollen and pigmented. Pigment is also found in KUPFER's cells. The liver cells contain only haemosiderin, not melanin. Pigmentation is most intense around the central veins.

Kidneys.—Pigmentation is much less marked. Changes may occur in the epithelium of the tubules, independent of the presence of parasites.

Bone Marrow.—Parasites and melanin, free, and in large mononuclear, leucocytes and macrophages are found. Crescents may be found here when absent or scanty elsewhere, as in the spleen and brain; it is consequently supposed that they principally develop here.

In cases of malaria of long standing the yellow marrow becomes red.

Stomach and Intestines.—In malaria with choleraic or haemorrhagic symptoms, parasites may abound in the capillaries of the villi.

CHRONIC MALARIA

Spleen.—As is well known, the spleen may in these cases fill the whole abdomen. Dilatation of the various lacunae occurs with a thickening of the splenic reticulum. The pigment tends to become deposited eventually in the connective tissue surrounding the follicles. The splenic septa become thickened.

Liver.—The pigment is found mainly in the *periphery* of the lobules, and pigment in the form of blocks in the perivascular connective tissue.

The capillaries are much dilated, and the epithelium contains blocks of pigment. Atrophy of the liver cells and their nuclei occurs.

Bone Marrow.—The marrow of the long bones is usually red, due to a large development of haematoblastic tissue. Normoblasts are common.

Pigment disappears rapidly from the bone marrow.

LITERATURE

Marchiafava and Bignami *Twentieth Century Practice of Medicine.* Malaria. Vol XIX. S Low, Marston and Co. *This comprehensive and learned treatise is incomparably the* best in the English language, dealing with all aspects of malaria and also blackwater fever.

Chapter XXV

BLACKWATER FEVER

Microscopical investigations in this disease are frequently negative as regards malaria parasites, but it is all important when the examination is made, as the following analysis of over one hundred cases *microscopically examined* shows :—

Parasites are present the day *before* the attack in ninety-five per cent. of cases.

Parasites are present the day *of* the attack in seventy per cent. of cases.

Parasites are present the day *after* the attack in twenty per cent. of cases.

In a series of cases examined by ourselves in British Central Africa we found malaria parasites *only in 12·5 per cent.*, but, as we have already shown, we have two further tests for a malarial infection :—

(1) The increase in the percentage of large mononuclear leucocytes.

(2) The presence of pigmented large mononuclear leucocytes.

By using these tests we were able to prove that 93·7 per cent., not 12·5 per cent., of our cases were due to a malarial infection.

Further, in the only case of blackwater fever seen by us *before* the onset of haemoglobinuria, *parasites were present* in abundance, afterwards they rapidly disappeared.

For the details of the proof we must refer to the original papers, where also the causative action of quinine is discussed. That quinine is the factor which, in the large majority of cases, determines the onset of haemoglobinuria appears to us equally certain.

Examination of the Blood in Blackwater Fever

1. Note the difficulty in obtaining a full-sized drop of blood.

2. Observe the 'thin' nature of the blood drop, its 'oily' nature, and the difficulty with which it adheres to the slide. These properties are best seen in severe cases.

3. Collect a specimen in a fine pipette and allow the serum to separate. Observe whether the serum is yellow (cholaemia) or reddish (haemoglobinuria), using the spectroscope if necessary.

4. To some of patient's serum add normal blood. Observe whether there is any haemolysis (using a haemocytometer if necessary).

5. Determine tonicity of patient's blood. Rate of coagulation approximately by placing several drops on a glass slide.

6. Count the red and white cells. The red cells are, as a rule, quite normal in shape.

7. Determine the amount of haemoglobin.

8. Make films every two hours if possible (*as early as possible*), noting accurately the time and temperature at which the films are made.

9. Examine films for parasites; if these are absent, search carefully several large films for

pigmented leucocytes, as these, as also in ordinary malaria, may require long search.

10. Make careful differential counts of the leucocytes, especially when the temperature is falling, as it is then that the mononuclear increase is most marked. When the temperature is raised (*e.g.*, 103° to 105°) the polynuclears may reach ninety per cent.

11. Observe presence of normoblasts, megaloblasts, various abnormal staining reactions, *e.g.*, polychromatophilia of the red cell, especially during recovery.

12. Make careful blood counts immediately before and after administering quinine when no haemoglobinuria results. According to PANSE* there may result a blood destruction due to the quinine, which does not shew itself as haemoglobinuria.

EXAMINATION OF THE URINE IN BLACKWATER FEVER

1. Before the attack (if possible) examine for albumen, urobilin, reducing bodies, etc.

2. Examine so-called 'high-coloured' urines. As a rule these do not shew bile pigment.

3. Examine urine during an attack for methaemoglobin (or haematin), oxyhaemoglobin, urobilin. bile pigment, bilirubin crystals, haeglobin casts, granular or hyaline casts, blood cells, etc.

4. Centrifugalize the urine. Examine the clear layer (as in 3), and make films of the sediment.

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*Panse. *Zeitschrift fur Hygiene*, 1903, s. 1.

The sediment may contain hyaline and granular casts stained with haemoglobin. The mass of the sediment, however, consists of masses of haemoglobin of a yellowish-red colour.

Post-Mortem Examination

1. Make smear preparations of spleen, *kidney, liver, bone marrow, brain, etc. Examine* for parasites and pigmented leucocytes. Parasites are generally absent, but pigmented leucocytes may occur in large numbers in the spleen. Fine pigment is also found in the liver in endothelial capillary cells (Fig. 66).

2. Cut sections, especially of brain tissue, as *parasites may be found here and nowhere else.*

Urobilinuria

As we have indicated elsewhere, the occurrence of urobilin may be an important indication in cases where a susceptibility to quinine haemoglobinuria exists thus in Murri's case, a girl had haemoglobinuria eight times between August 3, 1894, and April 6, 1895, following upon the administration eight times of small doses of quinine. From 1895 to 1897, the girl remained well. On March 27, 1897, she was given 0·5 grammes of quinine, to see whether her disposition to quinine poisoning still re mained. The result was fever, vomiting of bile, etc, albuminuria, peptonuria, and urobilinuria (not haemoglobinuria).

A. Plehn, in a recent paper, points out a peculiar property of the urine sometimes observed in blackwater cases. On boiling the

urine and allowing to stand for some time, a bright purple colour appears.

We have observed that blackwater urines made alkaline with potash, and then boiled produce a purple colour, giving the bands of haemochromogen (reduced haematin), shewing that the urine itself contained reducing bodies.

Whether PLEHN's purple colour is the same we cannot say.

THE KIDNEYS IN BLACKWATER

A. PLEHN, in the same paper, argues that:

1. There is not usually nephritis in blackwater (although this may exist as a complication).
2. Oedema is absent.
3. Cylinders are seldom present in the urine, red cell and leucocytes in uncomplicated cases are also absent.
4. Amorphous 'black-red pigment' only in severest cases. What PLEHN means by this is not clear. The remains of haemoglobinurous stromata, seen in severe cases, are stained a deep yellow.
5. Bile pigment is rare, if present there is generally true nephritis.
6. According to PLEHN, there is little alteration of the tubular epithelium, in our experience, however, this may be intense.
7. Sometimes insignificant sclerosis and small cell infiltration (but this depends on malaria).
8. In blackwater there is a *functional* disturbance of the kidneys (*c.p.* paroxysmal haemoglobinuria).

9. During anuria, no symptoms of renal colic as there would be if there were a blocking.

10. Absence of all uraemic symptoms proves that cessation of function of kidneys does not necessarily lead to uraemic symptoms.

LITERATURE.

KOCH. *Zeitschrift für Hygiene* (Bd. XXX, 1899, p. 295).

STEPHENS and CHRISTOPHERS *Reports to the Malarial Committee of the Royal Society* Harrison & Son, London

PANSE. *Zeitschrift für Hygiene* (1903, s 1)

Twentieth Century Dictionary of Medicine *Malaria* The fullest and best account in the English language

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Chapter XXVI

THE HAEMOCYTOZOA—*Continued*

The haemocytozoa, or endoglobular haematazoa, are divided by LAVERAN into three genera:

1. Genus *Haemamoeba.*
2. Genus *Piroplasma.*
3. Genus *Haemogregarina.*

The genus *haemamoeba* includes the malaria parasites with which we have already fully dealt. We now proceed to the other members of the genus, and then to the other two genera, which go to make up the endoglobular haematazoa.

GENUS HAEMAMOEBA

1. *H. Relicta (Proteosoma Grassii).*—Discovered by GRASSI in the blood of birds in Italy. In certain regions sparrows and goldfinches are commonly infected. Sparrows are frequently infected in India. In Africa numerous small birds were examined by us, but proteosoma was never found (only halteridium). Transmission from one bird to another by inoculation is readily effected. Canaries are extremely susceptible. Pigeons, among other birds, are immune. Birds that have recovered from an infection have acquired a well-marked immunity against a subsequent inoculation

The parasite is closely allied to the malaria parasite, and is especially suitable for the study of the exogenous mosquito cycle.

Endogenous Cycle (Fig. 67).—The parasite in its earliest stage is unpigmented. Coincident with growth a grain or two of pigment appears, and the characteristic property of the parasite shows itself, viz., the displacement of the nucleus of the red cell, so that the nucleus may take up a position at right angles and away from the normal one. All stages of development up to segmenting forms are found in the blood at the same time, so that no cycle of development can here be followed; nor is there any intermission in the clinical symptoms (temperature, etc.) of infected birds.

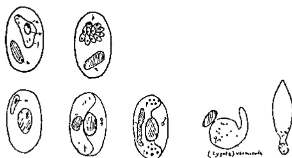


Fig. 67. (Upper line) Proteosoma shewing medium size Parasite and Segmenting Form (Lower line) Halteridium young form, Female and Male Gametes, and Vermicule

Exogenous Cycle.—Besides the asexual, sexual forms occur in the blood. They are spherical hyaline bodies of two varieties, characterized in stained specimens by the same general differences

which distinguish the male and female gametes of the malaria parasite.

(i) The male cell possesses a mass of compact chromatin and faintly staining protoplasm.

(ii) The female cell possesses but little chromatin, but stains deep blue (ROMANOWSKY).

Flagellation.—(i) This can be observed in a simple wet film preparation and in hanging drop preparations (p. 30), or

(ii) Use artificial serum (bird's serum, one part; salt solution, 0·6 per cent., nine parts), and to this add a trace of bird's blood. Make a series of hanging drops in moist chambers. Dry, fix, and stain, from time to time, according to stage of development, observed microscopically.

Further stages of development (vermiculi) have not been observed on the slide.

Development of Vermiculi.—(i) Determine what species of *Culex* is the suitable one for the process of development. *C. nemorosus* was used by KOCH, in Italy. *C. fatigans* is also a carrier.

(ii) Collect the *Culex* that have fed on sparrows, etc., roosting at night in trees. The *Culex* can be caught in large numbers in shaded drains, under bridges, in outhouses, etc., and excellent material is in this way easily got. Identify the species of *Culex* that is infected.

(iii) For the method of feeding mosquitoes on birds' blood, *vide* p. 101.

Twelve to fifteen hours.—Vermiculi in all stages of development are found in the stomach; a conical projection arises from the fertilized gamete. This gradually elongates, forming a long, curved, oval body, the complete vermiculus. The

protoplasm is vacuolated, and a nucleus (chromatin) is readily shown by staining (ROMANOWSKY).

The proteosoma vermiculi are larger and more slender than those of halteridium.

Development of Zygotes (one to two days).—The vermiculi have disappeared, but in the stomach wall are now found transparent, spherical, pigmented bodies.

Three to four days.—The zygotes have increased in size, and sporoblasts appear in their interior. In the larger forms, signs of further division are seen (striation), formation of sporozoits.

Development of Sporozoits (nine to ten days).—By this time the sporozoits have reached the salivary glands. Somewhat earlier they can still be found amidst the thoracic muscle. Earlier still, they can be pressed out of the ripe oocysts in the stomach wall. The sporozoits occupy chiefly the middle lobe of the gland (KOCH).

Black Spores are found in the larger zygotes. They also occur free in the thoracic region (or, possibly, in the gland substance). They are brownish black, curved, sausage-shaped bodies, suggesting a mycelial nature. It is believed by GRASSI that they are degenerated sporozoits, as they are found within the large sporoblast cysts. We have, however, found them in or about the salivary glands in *Myzomyia Rossii*.

2. *H. Danilewskyi* (Halteridium).—Occurs almost exclusively in the blood of 'passerine' birds. Pigeons are very commonly infected, also sparrows, finches, 'paddy' birds, etc.

The parasite is characterized by its peculiar curved halter shape, embracing the oval nucleus

of the red cell without any displacement of the latter (Fig. 67). Young forms are occasionally seen, but whether these are young sexual or asexual forms is not determined. Segmenting forms and those corresponding to an asexual cycle, as in proteosoma, are unknown.

Two varieties of parasites, the male and female gametes, are easily distinguished.

(i) Note that the male gamete has a clear hyaline appearance. On staining (ROMANOWSKY) a central mass of chromatin is distinguished, while the protoplasm is a faint blue. Five or more oval pigment grains are placed generally at either extremity.

(ii) In fresh specimens the female gamete is finely granular, and the pigment is frequently scattered throughout. On staining, a small amount of chromatin is shewn, while the protoplasm takes on a deep blue colour.

Flagellation.—Select an infected bird that shews numerous gametes in each field. Proceed in the same way as in proteosoma. The gametes first become spherical and then escape from the red cell. The pigment of the male gamete displays violent movement, and in a few minutes four to eight flagella are extended. The motion of these is at first so rapid that they cannot be distinguished, but the corpuscles in the neighbourhood are seen moving. In a few minutes one or more breaks off, and if, fortunately, a female gamete is in the same field, the loose flagellum (mikrogamete) can be seen entering the female. The pigment of the latter shews active movements at this stage.

Vermiculi.—The formation can readily be observed on the slide. A conical projection forms at one point of the fertilized gamete (copula). This elongates slowly and gets curved, forming an egg-shaped or spindle-shaped mass. The conical portion eventually separates, leaving behind the remains of the cell with the pigment. The vermiculus is thus at first unpigmented, but later again it is pigmented (KOCH). In the fresh specimen the protoplasm appears vacuolated, and has a nucleus which is readily stained by ROMANOWSKY stain.

Note that the vermiculus (or ookinet) shews forward, rotatory, and peristaltic motions. The further development of the vermiculi is completely unknown.

Post-mortem. — Pigment is found in the kidney, intestine, bone marrow, liver, and especially the spleen. The brain, on the contrary, is almost entirely free from it.

It is probable that the halteridia of all birds are not of the same species. Inoculation from one bird to another is extremely difficult, if not impossible. This may be due to the fact that the parasites in the blood are in all sexual forms. In monkeys we appear to have a parallel condition, viz., gametes only in the blood, the asexual forms being unknown.

3. *H. Kochi*.—These haemamoebae occur in monkeys. The forms usually met with are sexual forms. Asexual forms resembling young malaria parasites are very rare. Flagellation can be seen in fresh specimens. The parasites in the fresh film are spherical pale bodies

of the red cell without any displacement of the latter (Fig. 67). Young forms are occasionally seen, but whether these are young sexual or asexual forms is not determined. Segmenting forms and those corresponding to an asexual cycle, as in proteosoma, are unknown.

Two varieties of parasites, the male and female gametes, are easily distinguished.

(i) Note that the male gamete has a clear hyaline appearance. On staining (ROMANOWSKY) a central mass of chromatin is distinguished, while the protoplasm is a faint blue. Five or more oval pigment grains are placed generally at either extremity.

(ii) In fresh specimens the female gamete is finely granular, and the pigment is frequently scattered throughout. On staining, a small amount of chromatin is shewn, while the protoplasm takes on a deep blue colour.

Flagellation.—Select an infected bird that shews numerous gametes in each field. Proceed in the same way as in proteosoma. The gametes first become spherical and then escape from the red cell. The pigment of the male gamete displays violent movement, and in a few minutes four to eight flagella are extended. The motion of these is at first so rapid that they cannot be distinguished, but the corpuscles in the neighbourhood are seen moving. In a few minutes one or more breaks off, and if, fortunately, a female gamete is in the same field, the loose flagellum (mikrogamete) can be seen entering the female. The pigment of the latter shews active movements at this stage.

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3. *H. Kochi* —These haemamoebae occur in monkeys. The forms usually met with are sexual forms. Asexual forms resembling young malaria parasites are very *rare*. Flagellation can be seen in fresh specimens. The parasites in the fresh film are spherical pale bodies

containing brownish-yellow pigment. On staining, two types can be distinguished. The male (mikrogametocyte), pale homogeneous blue with much chromatin; the female, deep blue, granular, with little chromatin.

No temperature changes occur in the infected animals. The infection is not transmissible by inoculation (*cp.* halteridium).

Post-mortem.—The spleen is pigmented, the capsule thickened. Pigment also occurs in the marrow.*

PARASITES IN BATS

DIONISI has described in bats parasites which have a general resemblance to malarial parasites, but almost certainly have no real relation thereto. He distinguishes the following forms:—

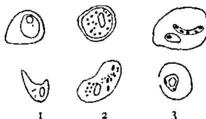


Fig 68. (1) *H. Murinus (above) medium size; (below) free form* (2) *H. Melanipherus (above) medium size; (below) large Ovoid form.* (3) *H. Vesperuginis (above) irregular form; (below) Ring form.* (*After* DIONISI)

4. *H. Melanipherus* (polychromophilus melanipherus).—This occurs in the blood of *Miniopterus Schreibersii*, and is so called on account of

*KOSSEL. *Zeitschrift fur Hygiene.* Bd. XXXII.

its staining reactions with ROMANOWSKY, and because it is pigmented. It somewhat resembles the quartan parasite (Fig. 68).

5. *H. Murinus* (polychromophilus murinus).—Found in the blood of *Vespertilio murinus*. It is also pigmented. It shews like the former a variety of 'polychrome' effects with ROMANOWSKY. In this, as in the former, DIONISI figures a great variety of forms (Fig. 68).

6. *H. vesperuginis* (achromaticus vesperuginis).—In the blood of *Vesperugo noctula*. The young forms resemble those of the malignant tertian parasite (Fig. 68). It occurs in large numbers in the blood, but forms no pigment during its development. It produces considerable anaemia and degenerative changes in the red cell.

How bats are infected is quite unknown.

The parasite 'find' differs according to whether the animal is hibernating or not.

7. [*H. bovis*].—Parasites in the blood of cattle, described by KOLLE in South Africa. They have a general resemblance to malaria parasites, but are quite distinct from *Piroplasma bovis*. They produce remittent fever and severe anaemia, but not haemoglobinuria. KOLLE also describes pigment in red cells (independently of parasites), but what this means is not clear.*

8. *H. Metchnikowi*.—Found in the blood of *Trionyx Indica*, or *Chitra Indica*, a large fresh-water tortoise, in many Indian rivers. All adult specimens of this tortoise from the Jumna were infected.

*KOLLE *Zeitschrift fur Hygiene* 1898

The parasite resembles *H. Danilewskyi* (halteridium), in that two forms are easily distinguished in the blood—(1) a hyaline form with large pigment grains, staining very slightly with methylene blue; (2) a granular form, with fine pigment, staining deeply with methylene blue. These forms correspond to the male and female gametes respectively. In one of SIMOND's figures it is interesting to observe a male and female gamete in the *same* red cell, which, so far as we know, has never been observed in the case of *H. Danilewskyi*. But besides these pigmented forms there are also found unpigmented forms,

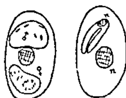


Fig 69 H Metchnikovi, Gametes and Vermicule

which have the typical gregarine look, that is to say, curved, worm-like bodies. The exact relationship of the haemogregarine to the haemaebae forms is not understood. SIMOND, however, points out that halteridium has a vermicule stage, and there is the possibility of the relationship being similar in this case (Fig. 69).

GENUS HAEMOGREGARINA

The haemogregarines are unpigmented unicellular organisms, which, at one stage of their development, have a worm-like form. They occur

as endoglobular parasites, and also as free forms in the plasma. The vermicule stage may be both endoglobular and free. The sexual and asexual cycles occur, as far as is known, in the same host. They occur in fish, amphibians, and reptiles, but not in mammals, and, unlike the gregarines, not in invertebrates. They are, so far as is known, non-pathogenic, and they cannot be transmitted by inoculation from one animal to another. The cycle of development, as far as it is known, will be described under the various species.



Fig 70 H Ranarum (or Drepanidium Ranarum) young form, Gametes free in the Plasma, and Fission forms in Spleen (Partly after Minchin)

1. *H. Ranarum (= Lankesterella Ranarum).* Found in the blood of *Rana Esculenta* (edible frog). This species includes, according to LAVERAN, two species, *H. princeps* and *H. monilis*, described by LABBÉ. Here, as in other species of haemogregarines, the sexual and asexual cycles occur in the same animal. The cycle of development is as follows:—

(1) *Sexuals Form or Schizonts*.—These are endoglobular, four to eight μ in length. Increase in size takes place, and eventually they become spherical and divide into a number of segments

(schizonts). According to some observers segmenting forms are only found in the spleen.

(ii) *Sexual Forms.*—Free in the plasma, twelve to fifteen μ long. These are male and female, and are characterized by the same general differences as other gametes; the male mikrogametocyte is slender and finely granular; the female makrogametocyte is fat and coarsely granular.

(iii) A mikrogamete in the form of a small mass of chromatin separates off and fertilizes the (now) makrogamete.

(iv) A zygote results, which is at first motile. This becomes encysted as the

(v) Oocyst, which is found in the *epithelial cells* of the intestine. This passes out eventually in the faeces of the frog. Sporoblasts are formed as in the malarial cycle, and from these result

(vi) *Sporozoits.*—These would gain access to a fresh frog which had swallowed an oocyst. HINTZE has shewn that frogs confined in pools are especially liable to infection.



Fig 71 H Splendens.—Adult form with Refractile Granules

2. *H. Splendens* (= *Dactylosoma Splendens*). —Found in the blood of *R. esculenta.*

The following forms are figured by LABBE (Fig. 71):—

(i) Amoeboid forms.

(ii) Forms resembling in shape a finger-glove.

(iii) Segmenting forms as in *Haemamoeba Relicta* (Proteosoma).

The protoplasm contains no pigment but refractile granules.

This differs from the typical development of haemogregarines, and it is probable that its position requires revision. According to HINTZE, it is a variety of *H. Ranarum*.

3. *H. Magna*.—Described by GRASSI and FELETTI in *R. esculenta*. MINCHIN thinks it may be the makrogamete of *H. Ranarum* or *H. monilis*.

4. *H. Riedyi*—Occurs in a Californian Salamander, *Batracheseps attenuatus*.

5. *H. Stepanowi*.—It is found in the tortoise, *Cistudo Europaea*. This may be taken as the type haemogregarine. It presents the following forms (Fig. 72) —

(i) Reniform parasites, ten to fourteen μ long. Curved and thickened at each end, granular, non-pigmented. Intermediate forms occur between this and the next developmental stage.

(ii) Vermicule forms, also endoglobular, but after examining a fresh specimen of blood for some time, free forms are seen thirty to forty μ long and three to four μ broad. These are actively motile, and constrictions can be seen travelling down their length during the motion. Young forms and reproductive forms are not seen in the circulation. These are

found in the liver. The reproductive forms are at first endoglobular, but later free. They occur as

(iii) Ovoid forms, ten to sixteen μ long by four to six μ broad, shewing as many as six nuclei (chromatin masses). The protoplasm finally segments and there is formed

(iv) An actively amoeboid young form.

The spores that are found in the kidneys of tortoises belong, according to LAVERAN, not to the haemogregarine at all, but are those of a *Myxosporidium (M. Danilewskyi)*.

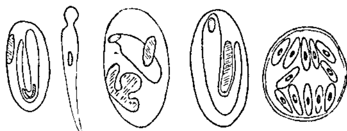


Fig 72. H. Stepanowi, Endoglobular and free Vermicules; H. Lacertarum, shewing disintegration of Nucleus of Red Cell; H. Lacazei, H Lacertarum, Cyst with Makromerozoits

6. *H. Lacertarum (=Karyolysus Lacertarum)* (Fig. 72).—Found in the blood of *Lacerta agilis*, *L. muralis*, and *L. ocellata*.

The parasite has a more compact form than some of the other haemogregarines. They exert a marked action on the red cells, which become much enlarged and anaemic, and, as the name of the species implies, a disintegrating

action on the nucleus is one of its effects. The nucleus is either pressed to the side or broken up into fragments.

The parasite in its endoglobular stage becomes encysted, and is then called a *cytocyst*. Further, it appears as if at this stage sexual differences appeared, for some of these cysts divide up into about a dozen *macromerozoits* (Fig. 72), while others divide up into twice as many or more *micromerozoits*. Corresponding to these we have free forms in the liver, twelve by three μ and eight by two μ respectively.

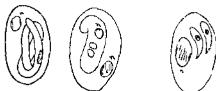


Fig 73. (1) *H Mesnili*, *shewing characteristic looped Vermicule*; (2) *H Laverani*, *shewing characteristic hooked Vermicule and two bright Granules* (*after* SIMOND), 3 *H bigemina in Blood of Blennies* (*after* MINCHIN)

7. *H. Lacazei* (= *Haemocytozoon claratum*). In the blood of lizards. The vermicules have a peculiar shape (Fig. 72). Here also cyst formation has been described in the spleen by LABBE.

8. *H. Mesnili*.—In the blood of a tortoise, *Emys tectum* (Fig. 73).

Amoeboid forms, reniform, and vermicule forms occur. Besides these, free merozoits, but their origin is obscure. The form of the vermicule is characteristic at one stage of its development.

9. *H. Laverani.*—In the blood of Indian tortoises, *Cryptopus granosus.* Similar forms occur to those of the last species. The vermicule is characterized by a blunt hook-like appendage, and the presence of two bright granules.

The parasite is endoglobular in all its stages.

10. *H. Bigemina.*—Discovered by Laveran in the blood of blennies. A vermicule form occurs free in the plasma.

The endoglobular parasite divides by simple binary fission. In fishes we also have *H. delagei* in two species of ray and *H. simondi* in the sole.

These must suffice to give an idea of the characteristics of these parasites, but they by no means exhaust the total, for haemogregarines have been described in thirty-one species of snakes, seven lizards, three crocodiles, eleven tortoises and turtles, so that ample material for study exists in the tropics. The subject is at present, however, in considerable confusion, and much further work is required.

Genus Piroplasma

SPECIES		HOST
P. bovis	- -	Cattle (Texas fever organism)
P. Canis	- -	Dogs (Italy, Senegal, France)
P. Ovis	- -	Sheep (Italy, Roumania)
P. Equi	- -	Horse (S. Africa)
P. Hominis	- -	Man (producing 'spotted fever')

1. *P. bovis.*—The parasite of Texas fever of cattle clearly does not belong to the proper group of malaria parasites, although it occurs in the red cell. It forms no pigment, and differs in its development from the malaria parasite (Koch).

The parasites are two to four μ in length, one to two μ in width. Two forms occur in the circulation—(i) a spherical or ovoid form, (ii) a piriform parasite in pairs. These are characteristic, and give the name. Intermediate stages between (i) and (ii) occur. The spherical forms show a chromatic particle, and closely resemble 'young rings.' The chromatic body (nucleus) divides into two portions, one going to each end; the parasite elongates, and by this means the piriform body is got. The piriform parasites are two to three μ long and about one μ in diameter.

The number of parasites in the peripheral circulation is proportionate to the severity of the disease—one to two per cent. of corpuscles are infected, at the end of the disease five to ten per cent. (or even twenty-five to thirty per cent.) The number in the blood is not so great as the number in the spleen (ten per cent.), liver (thirty per cent.), and especially kidneys (eighty per cent.)

Free parasites are found in the blood in the later stages of the disease, but especially in the kidneys.

Post-mortem.—Haemorrhagic oedema about the stomach, kidneys, and retroperitoneal tissue. Intense hyperaemia of the spleen and kidneys, the latter are nearly black. Haemorrhagic erosions. Ulcers in various portions of the alimentary canal. Ecchymoses in pelvis of kidney.

Transmission by Ticks.—According to MOTAS (Bucharest), *Piroplasma ovis* can be transmitted by transferring *adult* ticks from an infected to a non-infected animal. He did not succeed in transmitting the disease by larvae or nymphae developed from ticks taken from infected animals

This is in direct opposition to the classical researches of Smith and Kilborne on *Piroplasma bovis*, corroborated by Koch. Smith and Kilborne hatched young ticks from the eggs of ticks that dropped off infected cattle. It is these *young* ticks that communicate the disease.

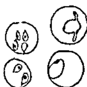


Fig. 74. Piroplasma Canis (left), typical Piriform Parasites, (right) Amoeboid forms

2. *Piroplasma canis.*—The parasite is morphologically identical with *Piroplasma bovis.* It is a strictly specific parasite, and has not been transferred to any other animal than the dog. Native dogs in the tropics may harbour the parasite without shewing any symptoms. The disease exists in France, Italy, South Africa, etc. In the chronic forms the parasite is rare in the circulation, but in the acute form with high fever, icterus, and haemoglobinuria, the parasite (typical pirosoma form) is found with great ease in the blood. Four to six parasites often occur in each cell. Kidney blood post-mortem is extremely rich in parasites. Young dogs, two to twelve weeks old, are the most easily infected by intravenous injection. The tick, *Demacentor reticulatus*, is supposed to convey infection in Europe, and *Haemaphysalis laechi* in South Africa. Both these ticks appear to pass their larval stages on other hosts than the dog.

3. *Piroplasma ovis.*—The disease in Hungary is known as càrceag. BABES considers that the organism forms a connecting link between the bacteria and protozoa. Sheep that have recovered have a marked immunity.*

4. *P. [Kochi]* —'African Coast Fever,' described by KOCH, is an exceedingly virulent form of piroplasma infection in Rhodesia and South Africa, eighty to ninety per cent. of infected cattle die. A peculiarity of the disease is that the anaemia is slight and, correspondingly, haemoglobinuria rare. The parasite is smaller than that of the piroplasma of Texas fever. The parasites are disc-shaped or leaf-shaped, and as the disease progresses may be found in almost every cell. Pear-shaped organisms are rare. It is suspected that *Rhipicephalus decoloratus*, or the blue tick, transmits the disease, for this tick transmits also Texas fever in South Africa.



Fig 75 Atypical forms of Piroplasma (After LAVERAN)

It is in this form of bovine piroplasma especially that atypical forms have been described by THEILER, and subsequently more fully by LAVERAN.

(i) Forms resembling straight or curved bacilli, one to three μ long. They are thicker at one end, which contains a chromatin particle. One to four may occur in the same cell.

*MOTAS *Soc. de Biol.* 1903, p. 1,523

(ii) Forms resembling cocci, singly or in pairs. Two to four may occur in the same cell.

It is important to note that together with the atypical forms typical forms are always found, though these latter may be rare.

These atypical forms occur in the severe cases Similar cocci-like forms, described by SMITH and KILBORNE in Texas fever, occurred in the *slight* cases. The post-mortem lesions, characteristic of this form of pirosomal disease, are local infarcts in various organs.

5. *Piroplasma hominis.*—This species of piroplasma is responsible for the disease known as 'spotted fever' (fleck-typhus), occurring in Montana and Idaho, U.S.A., and also in Egypt. As the name implies, there accompanies the fever an eruption of spots. The mortality in the United States is as high as seventy to eighty per cent. It is much less in the cases described in Alexandria, Egypt. It is possible that they are not the same diseases, and the subject requires elucidation. In the Montana disease, piriform, ring-shaped, and cocci-like forms occur.

6. *Piroplasma Equi* (LAVERAN).—Found in horses in South Africa.

TICKS

Life History.—The female, after satiating herself with blood, falls to the ground and, in a few days or weeks, lays eggs.

Eggs.—The eggs are laid in masses of several thousands (*Ixodidae*), of some hundreds (*Argasidae*). The process lasts about a week. They are small, oval, opaque bodies. They may take weeks or months to hatch out. From the eggs is developed—

The Larva.—These are hexapod. They cling to blades of grass, etc., and may do so for several months before attacking a host. The larval stage lasts six to ten days. The moult then takes place, and there emerges from the skin—

The Nymph.—These are octopod. They resemble adult females. They have respiratory stigmata, but no sexual organs. The nymphal stage lasts seven to ten days. The nymph moults, and there emerges—

The Adult.—These again attach themselves to the host, and in a few days copulation takes place. The female gradually distends and remains attached for about *nine* to *eleven* days. The female then drops off. What the male does is uncertain. The female's whole cycle *on the host* is thus twenty-two to thirty-one days. The *entire* life cycle takes, probably, about two to three months.



Fig 76 Eggs, Larva, and Adult Tick (*After* MANSON)

The dimensions of the various stages of *I. Ricinus* are:—

Eggs	..	0·4 by 0·3 millimetres.
Larva		0·6 by 0·4 millimetres.
Nymph	.	*1-3* by *0·6-2* millimetres.
Adult .		2·5 by 1·5 (male) millimetres.
Adult	. .	10-11 by 6·7 (female full grown) millimetres.

Ticks have a predilection for certain hosts, but the same tick may be found on different animals, and, further, the larval and adult stages may be passed on different animals.

Anatomy

1. *The Rostrum*, capitulum or head, is the small anterior projecting portion, it is joined on by a short neck to the scutum; on its ventral surface is seen—

2. *The Labium* or hypostome, a bi-laterally symmetrical structure, furnished with a number of teeth directed backwards. The number of rows of teeth and their disposition are very important in classification.

3. *The Mandibles* lie dorsally to the labium. The terminal portion (digit) terminates in two or three processes, apophyses, which bear hooked teeth directed backwards. They are important in classification.

4. *The Mandibular Sheath* lies above the mandibles. The anterior extremity is notched corresponding to the two halves of the sheath. (2), (3), and (4), form the piercing organ or *haustellum*.

5. *The Palpi* are four-jointed and form a kind of sheath for the haustellum. The shape of the palpi, their spines and processes are of the greatest importance in classification.

6. *The Scutum* is a dorsal structure, situated behind the base of of the rostrum. It is a hard leathery plate. In the male it practically covers *the whole* of the dorsum. In the female it is confined to a roughly triangular anterior portion of the dorsum. The males are thus

readily distinguished from the females. It is *absent in the Argasidae.*

7. *The Porose areas* are dorsal structures forming two oval depressions one on each side of the middle line at the base of the rostrum. They are most conspicuous in the female, but exist in both sexes.

8. *The Eyes*, not always present, are small, almost globular structures, situated laterally at the margin of the scutum in the *Ixodidae*, or as punctiform structures on the supracoxal fold of the first leg in the *Argasidae*.

9. *The Stigmata.*—Situated ventrally and laterally behind the level of the fourth legs in the *Ixodidae* open into a stigmal plate or peritreme. In the *Argasidae* they lie between the third and fourth legs. The shape of the plates are important in classification.

10. *The Anus* is a little way in front of the posterior ventral margin. It has a valve.

11. *The Anal plates* or *clypei*, four in number, on either side of the anus, in the male. They are used for classifying. Not always present.

12. *The genital orifice* is in the middle line, a little way behind the rostrum.

13. *The Legs* are six in the larva, eight in the nymphs and adult The claws have ventrally a well-marked pad or *pulvillum.* The coxa (with which the trochanter articulates) may have spines or teeth or larger 'shields.' These are used in classification.

Classification of Ticks

Ticks are divided into two families—

1. *Argasidae*, scutum absent.
2. *Ixodidae*, scutum present.

The *Ixodidae* are divided into two sub-families—

1. *Rhipicephalinae.*—Palpi not longer than broad, rostrum short. Anterior portion of body emarginate to receive the rostrum.
2. *Ixodidae.*—Palpi longer than broad, rostrum long. Anterior portion of body straight or emarginate.

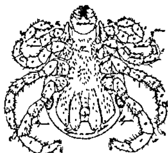


Fig 77 Tick under surface, shewing Anatomy and parts used for classification (*After* SALMON *and* STILES)

The *Rhipicephalinae* are the most important from our standpoint, as to the genus *Rhipicephalus* belong most of the ticks that are known to transmit parasites. The various genera are *Rhipicephalus*, *[Boophilus]*, *Haemaphysalis*, and *Dermacentor.*

GENUS RHIPICEPHALUS

Eyes present. Base of rostrum hexagonal (dorsally), forming on each side a projecting angle. Palpi short and broad. Stigmata, comma-shaped; clypei, two pairs in the male. Coxae i, two large teeth. The genus includes nearly thirty species. Some of these, including the carriers of piroplasma, we may classify in the following way :—

Classification of part of Genus Rhipicephalus

Species	Furrows of Scutum	Labium	Mandibles	Scutum in Male	'Tail' in Male	Remarks
R Annulatus	Extend to post lat margin	Eight rows of teeth	Int apophysis bicuspid	Extends to post margin	Absent	= *B. Annulatus* = *B. Bovis* Transmits American Texas Fever
R Caudatus	ditto	Ten rows	—	ditto	Distinct	
R Evertsi	ditto	Six rows	Tricuspid	Not extending to post margin	Small	= Red Tick, South Africa
*R decoloratus**	ditto	Six rows	Bicuspid and a rounded process	Extending to post margin	Distinct	Transmits Texas Fever in South Africa. = Black Tick of S Africa and Rhodesia
R Australis	ditto	Eight rows	Tricuspid and a rounded process	ditto	—	Transmits Texas Fever in Australia
R Sanguineus	ditto Scutum with unequal punctations	Six rows	Tricuspid	Not extending	—	Europe, Africa, America, commonest tick in Rhodesia
R Pulchellus	Become obsolete in middle Scutum white ♀ Black and white ♂	—	—	—	—	Zanzibar

* Koch describes a tick closely resembling this, but with eight rows of teeth on the labium along the East Coast of Africa.
This species and *R decoloratus* are possibly the transmitters of 'African Coast Fever' of Cattle

Genus Boophilus

Not admitted by Neumann as a genus.

Genus Haemaphysalis

Eyes wanting. Base of rostrum rectangular, twice as long as broad, palpi conical. Second segment of palpi has a well-marked lateral conical projection. Stigmata comma-shaped or circular. Anal shields absent in male. Coxa i not bifid. Coxa iv in male, a well-marked spur. There are about twenty-six species.

H. Leachi (South Africa).—The dog is the usual host. Possibly transmits *Piroplasma canis*, occurs also on cattle.

Labium.—Four rows of teeth in ♀, five rows in ♂.

Palpi.—Dorsal surface as broad as long. Second palpal segment has a sharp lateral spine.

Coxa iv.—Has a tuberosity.

Genus Dermacentor

Eyes present. Base of rostrum broader than long. Palpi short and thick. Stigmata, comma-shaped. Anal shields absent in male. Coxa i bidentate in ♂ and ♀. Scutum ornamented. About twenty-four species.

D. Electus is the American dog tick.

The subfamily *Ixodinae* consists of five genera, *Ixodes*, *Eschatocephalus*, *Aponomma*, *Amblyomma* and *Hyalomma*.

Genus Ixodes

Eyes absent. Palpi long. Tarsi without terminal spurs. Anal groove surrounds anus anteriorly and opens posteriorly. Scutum in male does not cover the body laterally and posteriorly. Stigmata oval in ♂, circular in ♀. Male ventrally covered with six shields; two lateral, embracing the origins of the legs and the stigmata; one median, between the genital opening and the anus; two on each side of the anus (perianal), and one triangular posterior shield, carrying the anal orifice at its anterior corner. Female has dorsally three longitudinal grooves on the abdomen, ventrally two bell-shaped grooves, the first has its apex at the vulva, the second at the anus. There are a large number of species.

1. *I. ricinus.*—The castor-bean tick is common on sheep, goats, cattle: Europe.
2. *I. hexagonus.*—The European dog tick.

Genus Eschatocephalus

Eyes wanting. Rostrum long. Palpi piriform ♂, claviform ♀. Anal grooves as in *Ixodes.* Stigmata circular in both sexes. Legs very long. Dorsal and ventral chitinous thickenings in the male; fine grooves in the female. There are seven species.

Genus Aponomma

Eyes wanting. Anal groove surrounds anus posteriorly, and opens anteriorly. Anal plates absent. Base of rostrum pentagonal. Scutum

covers the dorsum entirely; usually marked with green spots. Stigmata, comma-shaped. Female, scutum shorter than broad, three green spots. The species are parasitic on reptiles.

Genus Amblyomma

Eyes present, conspicuous. Anal groove as in *Aponomma*, anal plates absent. Rostrum long. Scutum often has coloured designs. Stigmata usually triangular, nearly always eleven posterior marginal festoons in the male. There are over eighty species.

A. variegatum.—Is frequent on cattle in Rhodesia.

Genus Hyalomma

Eyes present, conspicuous. Rostrum long. Anal groove opens anteriorly. Body elongate oval. Colour deep-brown. Male, two pairs of ventral shields, two perianal, large, triangular, and two small external. Scutum covering nearly the whole of the dorsum. Crenellated or festooned posteriorly. Male, stigmata comma-shaped, with a long tail; female, stigmata with a short tail. Three species only.

H. Aegyptium.—Attacks cattle especially, also dogs and cats. Occurs in Egypt, North and South Africa.

Argasidae.—Scutum absent, rostrum inferior (except in larva). Stigmata between third and fourth legs. Pulvillum of tarsi wanting in adult. Palpi, free, short, filiform, four segments. Tegument leathery, without dorsal or ventral shields. Sexual dimorphism not marked.

Genus Argas

Eyes absent. Rostrum, which is concealed by the cephalo-thorax, is situated at least its own length behind the anterior margin. No projecting hood. Body oval or orbicular. The species are nocturnal in their habits, infest birds mainly. There are eleven species. A species of Argas is the transmitter of 'spirillar fever' of poultry.

A. reflexus infests pigeons, European.

A. *persicus* = Garib-Guez of Persia. The bite is said to produce severe local and constitutional effects.

A. tholozani = Kéné of Persians, similar effects ascribed to it.

Genus Ornithodorus

Eyes present or absent. Rostrum hidden under a projecting beak (hood), close to the anterior margin of the body, so that the tips of the palpi are visible from above. Lateral borders of body generally straight, sometimes concave, tegument mammillated.

O. moubata = 'Garrapata,' tick of Tete on the Zambesi. The bite is said to occasion severe local and general disturbances. It is the cause of 'tick fever' in Uganda (Christy).

YELLOW FEVER

Myxococcidium Stegomyiae

The following is a brief summary of the work done by the Americans (Parker, Beyer, and Pothier) on the parasite of yellow fever.

LITERATURE

1. *Cattle Ticks of the United States* Salmon and Stiles, Washington, 1902. An excellent compendium on ticks, many illustrations. Price, a few shillings.

2. L. G. Neumann. Révision de la famille des Ixodidés, *Mémoires. Soc. Zool. France.* Complete monographs.

3. *Texas Fever.* Theobald Smith and F. L. Kilborne, 1893. Washington. Bulletin No. 1.

4. Wasielewski *Sporozoenkunde.*

5. *The Sporozoa.* Minchin in Lankester's Zoology.

6. *A Monograph of the Tsetse Flies.* Austen (Nat. Hist Brit. Mus.)

Chapter XXVII

THE TRYPANOSOMIDAE

TRYPANOSOMATA AND TRYPANOPLASMATA

The *Trypanosomidae* comprise two genera—(1) *Trypanosoma*, (2) *Trypanoplasma*. The genus *Trypanosoma* is characterized by the possession of a longitudinal undulating membrane, the thickened border of which takes its origin posteriorly from a centrosome, and terminates anteriorly in a free flagellum. Division takes place longitudinally. The genus *Trypanoplasma* has two flagella, one anterior the other posterior. Both arise from one centrosome; the anterior forms the thickened border of the undulating membrane; the posterior flagellum curves around the posterior end of the parasite, and then is prolonged into a flagellum about equal in length to the anterior one.

The *Trypanosomidae* occur in fish, amphibia, reptiles, birds, and mammals. Most of these are very incompletely known, and it is only some species in mammals that have been at all closely studied. We may enumerate the following species:—

Trypanosoma rotatorium (synonym = *Trypanosoma Sanguinis* (GRUBY).—In the blood of frogs (*Rana esculenta*, *Rana temporaria*, *Hyla arborea*, etc.), forty to eighty μ long, five to ten μ

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broad. Flagellum ten to twelve μ long. Surface of the body striated longitudinally (Fig. 79 and Pl. II, fig. 3).

Ts. Carassii (MITRAPHANOW).—Besides the forms with undulating membrane and flagellum, disk-like forms are described. In the blood of fish (*Carassius vulgaris*); in the tench (*Tinca vulgaris*). Also described in the blood of the stickleback, pike, etc. (Fig. 79).

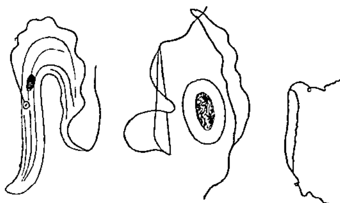


Fig. 79. T. Rotatorium, T. Cobitis, T. Carassii, Tp. Danilewski. Left to right. (After LAVERAN *and* MESNIL, MITRAPHANOW *and* DANILEWSKI)

Ts. Eberthi (KENT).—In the guts of fowls, ducks, geese, especially in the caecum and ileum. This is not a blood parasite so far as is known.

Ts. Cobitis (MITRAPHANOW).—In the blood of the mud-fish (*Cobitis fossilis*). Thirty to forty μ long, one to two μ broad. Flagellum ten to fifteen μ. It is long and thin. Forms without an undulating membrane are described, and also without a flagellum (Fig. 79).

Ts. soleae (LAVERAN).—In the blood of the sole.

It is, however, the trypanosomes of mammals that are of the greatest importance, producing as they do well-known and fatal diseases. Not all trypanosomes of mammals are, however, pathogenic. Trypanosomes are bodies easily detected in fresh blood with a one-sixth or one-seventh lens. They are actively motile, and may be seen displacing the red cells by their motions. As they come to rest the undulating membrane and flagellum are visible. They are bodies about twenty μ long. In stained specimens (ROMANOWSKY) an oval nucleus lies about the middle of its length, and near the blunt posterior end a small stained particle is clearly



Fig. 80. T. Lewisi.—(1) Dividing form with two centrosomes. (2) Adult form. (3) Young forms resulting from division (fresh preparation)

seen, the centrosome. From this, in certain species at any rate, the flagellum starts, and can be seen as a distinct wavy thick red line extending the whole length of the organism and continued beyond as the long (anterior) free flagellum. The portion (unstained) between this external wavy margin and the blue stained body of the organism is the undulating membrane (Fig. 80).

(c) Branched hairs on the upper surface only of the *arista* (the arista is an appendage of the terminal segment of the antenna) (*vide* Fig. 82).

(d) Male genitalia (hypopygium) characteristic.

(e) Membrane of wing grooved, not smooth, as in other genera.

(f) Wings closed flat over one another like a scizzors.

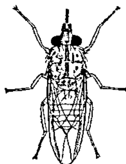


Fig. 83. *Glossina, shewing scissors position of Wings when at rest,* × 2 (*After* AUSTEN)

2. *Stomoxys* :

(a) Greyish flies, with black markings (smaller than *glossina*), found on men and cattle.

(b) Palpi exceedingly slender and short, not protecting the proboscis.

(c) Fleshy labella, small but visible.

(d) *Arista* distally forms a fine hair.
(e) Wings diverge widely. S. *Calcitrans* is a common European species.

3. *Haematobia* :
   (a) Small mottled flies, *e.g.*, 'horn fly,' of United States.
   (b) Palpi distinctly shorter than proboscis, expanded at the tips.
   (c) Tip of proboscis bears a few hairs.
   (d) The modified flesh labella easily visible.
   (e) The arista has hairs on under and upper surface.



Fig. 84. Stomoxys, shewing resting position of Wings. × 2 (After Austen)

4. *Lyperosia* .
   (a) They occur in great numbers on, for instance, camels.
   (b) By some regarded as a sub-genus of *Haematobia.*
   (c) Palpi form a complete sheath for the proboscis, and flattened as in *Glossina.*
   (d) Palpi not expanded at the tip.
   (e) Closely allied to *Glossina*, but differing in the wings.

5. *Beccarimyia* :
   (a) Proboscis resembles that of *Haematobia.*

(b) Palpi not expanded at the tip.
(c) Arista feathered only on upper surface.
(d) Has a prominent *Epistoma* (thus differing from other genera). [The tip of the Epistoma is the point from which the total length to the tip of the abdomen is measured].
(e) First posterior cell of the wing closed before the margin (in *Glossina* it is open).

Other blood-sucking flies that may be confused with the tsetse flies belong to a different family, viz., *Tabanidae* (horse flies, clegs, or gad flies). In this family those resembling tsetse are the genera.—



Fig. 85 Haematapota, shewing resting position of Wings, × 2 (After AUSTEN)

1. *Hematapota*:
   (a) They are about the same size as large tsetse flies—eleven mm. long.
   (b) The abdomen is not banded.
   (c) The wings diverge at the tip but meet at the base (*vide* Fig. 85).
   (d) *The antennae project horizontally forwards and are conspicuous.*
   (e) The grey small *Haematapota* of English lanes in summer is a familiar species.

2. *Pangonia:*
   (a) Characterized by the extreme length of the proboscis in some species, three to four times the length of the body.
   (b) The 'Seroot' fly of Nubia is a species of *Pangonia*.
   (c) Antennae projecting (family character).

Genus Glossina

Abdomen generally, but not always, has pale but well-marked dark-brown bands interrupted in the middle.

1. Dull-coloured, brownish flies, seven to twelve mm. long (excluding proboscis and wings).

2. Wings in resting position, closed flat, one over the other, scizzors-like, projecting beyond the abdomen.

3. Proboscis enclosed in palpi, projecting horizontally in front.

4. Base of proboscis suddenly expanded into a large onion-shaped bulb.

5. Arista feathered on upper side only.

6. Male genitalia (hypopygium) highly characteristic, oval and tumid, with a vulviform median groove (anus) running from anterior margin to beyond the middle. Sex easily distinguished by this mark.

7. Wings absolutely characteristic, especially in the course of the *fourth longitudinal vein* (*vide* Fig. 82, iv). The anterior transverse vein is

very oblique. The bend in the course of the fourth vein, before it meets the anterior transverse vein, is absolutely diagnostic.

Larva and Pupa.—According to BRUCE, tsetse flies (or at least one species) do not lay eggs, but extrude a yellow-coloured larva. After a few hours this changes into a pupa. The pupa is six mm. long and three mm. broad. It consists of twelve segments. The *twelfth segment* is produced into two large lips, enclosing a pit, the site of the respiratory stigmata in the larva. At the anterior end is a longitudinal groove, through which the fly eventually emerges.

CLASSIFICATION OF SPECIES

(1) Hind tarsi entirely dark.

Gl. palpalis.—Darkest of all species of *Glossina.* Third joint of antenna dusky-brown to cinereous black.

Gl. pallicera.—Third joint of antenna orange-buff. Front in both sexes *narrower* than in *Gl. palpalis.* In ♂ the arista is stouter and longer than that in *Gl. palpalis.*

(2) Hind tarsi not entirely dark.

Small species, length not exceeding ten-and-a-half mm. Last two joints of front and middle tarsi have sharply defined dark-brown tips.

Gl. morsitans.—

1. Smaller than *G. longipalpis.*
2. Head narrower.
3. Front paler and wider.
4. Eyes in ♂ and ♀ distinctly converging towards vertex.

5. Abdominal bands less deep, pale hind margins of segments therefore deeper.
6. Hypopygium in ♂ larger, paler, somewhat more oval in outline, and clothed with fewer hairs.
7. Tip of ♂ abdomen less hairy laterally.
8. Bristles on sixth segment in ♂ stouter and more conspicuous than in *longipalpis*.

Gl. longipalpis: Small Species.—Last two joints of front and middle tarsi entirely pale.

G. pallidipes.—*Large species.* Length, at least ten-and-a-half mm. (in this respect they contrast markedly with the other small species).

G. longipennis.—

1. Thorax with four sharply defined dark-brown oval spots.
2. Ocellar spot, dark-brown, *very conspicuous* compared with the body.
3. Proboscis shorter than in *G. fusca*, and *relatively* shorter, compared with the body, than in any other species.
4. In both sexes the *front* is broader than in *Gl. fusca.*

G. fusca.—Thorax without spots.

T. Evansi.—The trypanosome of Surra. A common disease in many parts of India, *e.g.*, Bombay, at certain seasons especially, though probably always in a latent condition. It is possible that its increase at a particular time is associated with the prevalence of a biting fly. Surra is characterized by a similar train of symptoms to those of Ngana. ROGERS states that the disease in India is conveyed by *Tabanidae* (horse

flies). If this is so, the probability of Ngana being also conveyed by other biting flies than the genus *Glossina* seems likely.

Whether the 'surra' of camels in India is produced by the same trypanosome there is no evidence to shew.

LAVERAN and MESNIL, who have recently been able to make a comparison of *T. Brucei* and *T. Evansi*, state that *T. Brucei* is shorter and more compact than *T. Evansi*. The movements of *T. Brucei* are also less extensive. The posterior end of *T. Brucei* is also blunter than that of *T. Evansi*. The free portion of the flagellum is shorter in *T. Brucei* than *T. Evansi*, and the protoplasm of *T. Brucei* has more numerous and larger granules than that of *T. Evansi*. The nuclei and the centrosomes are morphologically indistinguishable. Further, the mean length of *T. Brucei* is less than that of *T. Evansi*, and the width of *T. Brucei* is greater. The distinction between Surra and Ngana is, however, best proved by the fact that an animal immunized against Ngana is yet susceptible to inoculation with Surra.'

Trypanosoma Equinum (Mal de Caderas).—In Central and South America. A disease affecting horses.

The symptoms—remittent fever, oedema, wasting—resemble those of Ngana and Surra. Most characteristic is the paralysis of the hind legs, from which the disease takes its name.

It runs a chronic course, two to six months. In donkeys six to twelve months. There is occasionally haemoglobinuria.

Mice, rats, rabbits, dogs, etc. (guinea pigs rarely), are susceptible. Incubation period, five to eight days. Horned cattle are refractory.

It is thought that the infection is transmitted by a biting fly *(Stomoxys calcitrans)*. The parasite morphologically resembles *T. Brucei*. In the latter the centrosome is, however, larger. In *T. Equinum* it is so small that its existence has been denied. Moreover, an animal immunized against *T. Equinum* is still susceptible to *T. Brucei*.

T. Equiperdum (Fig. 81).—This trypanosome is the cause of the disease among horses in Algeria known as *Dourine*. In asses the symptoms are slight. In horses, and especially stallions, the symptoms are much more marked. It is conveyed, as far as is known, under natural conditions by 'coitus' only, and not by means of flies.

In eleven to twenty days after coitus, oedematous swellings of the genitalia appear.

In forty to fifty days characteristic 'plaques' on the skin. These are very occasionally absent, as in asses, but when present are pathognomonic. These 'plaques' last only one to eight days. Around these there is oedema. The animals become anaemic, complete paraplegia sets in, and death in two to ten months.

Trypanosomes are most easily found in the 'plaques,' with difficulty in the blood.

Post-Mortem.—There is inflammation of the urogenital mucosa, and in two cases areas of softening have been found in the spinal cord. Ruminants are refractory (to *T. Brucei* they are very susceptible). Dogs which have been immunized against *T. Equiperdum* yet succumb to *T. Brucei*, so that Dourine and Ngana appear to be distinct.

T. Gambiense (DUTTON).—This, the first human trypanosome to be described, was discovered by DUTTON in the blood of a European in the Gambia. The clinical symptoms of the case were:—

(1) Irregular relapsing fever.
(2) Oedema, especially about the eyes.
(3) Congestion of the skin.
(4) Erythematous patches, associated with thickening of the skin.
(5) Increased pulse and respirations. Loss of flesh.

The trypanosomes are generally scanty in the blood. They are transferable to monkeys, where they persist in small numbers for some months; also to white rats. Careful examinations of the blood by centrifugalization are necessary for their detection in most of these animals.

How conveyed to man there is no evidence to show (*vide* Plate I).

SLEEPING SICKNESS (TRYPANOSOME)

T. Ugandense (CASTELLANI).—Found in the cerebro-spinal fluid obtained by lumbar puncture by CASTELLANI, in seventy per cent. of cases. Later, BRUCE found it in thirty-eight cases in all, and in the blood, in twelve out of thirteen cases. Centrifugalization is necessary.

There are morphological differences between *T. Ugandense* and *T. Gambiense* according to CASTELLANI. The former has a more rounded posterior extremity. The centrosome is nearer the extremity and outside the vacuole, which is larger (these differences, however, may not be constant).

Symptoms.—Perhaps most noteworthy in comparing it with other forms of trypanosomiasis, if indeed the disease is due to this trypanosome, are:—

1. A puffiness about the face.
2. An enlargement, especially of the cervical lymphatics (this, however, Dr. CHRISTY informs us is not an essential character).
3. An itchy papulo-vesicular eruption of the skin.

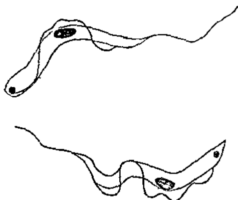


Fig. 86. T. Ugandense (CASTELLANI), *above*, *T. Gambiense* (DUTTON), *below*. (*After* CASTELLANI)

It should be remembered that in eighty per cent. of fatal cases a variety of streptococcus pyogenes was cultivated from the organs. Whether or no this is to be regarded as a terminal infection, and what the relation of the trypanosome to the disease may be, remains to be seen.

BRUCE, who has continued his work, finds that the distribution of the disease in Uganda

is identical with that of *Glossina palpalis*, and that the *trypanosome* can be conveyed to monkeys by means of *Glossina*. To CHRISTY belongs the credit of having previously shewn that the disease could not be due to *F. perstans*, as had been supposed.



Fig. 87 *Hippobosca Rufipes, left* × 2—*right, natural size* (*After* THEILER)

T. Theileri.—This is found in the blood of cattle in the Transvaal, subject to a disease known as 'gal ziekte,' *i.e.* gall sickness. Length, thirty to sixty-five μ, width, two to four μ.

THEILER has shewn since that a biting fly, *Hippobosca rufipes*, transmits the disease.

T. Transvaliense.—Found in the blood of oxen, eighteen to fifty μ long by four to six μ broad. The centrosome of this trypanosome almost touches the nucleus. The undulating *membrane is consequently little developed.*

Aino.—Is the native name for a disease in Somaliland, affecting camels, horses, asses, mules. It is caused by a trypanosome, and is exceedingly fatal to camels. Man, dog, etc., in these regions are not naturally infected.

Dimensions of Trypanosomata Found in Mammals

1.	*T. Lewisii* -	24-25 μ by 1-4 μ
2.	*T. Brucei* -	25-30 μ by 1·5-2·5 μ
3.	*T. Equiperdum* -	18-26 μ by 2-2·5 μ
4.	*T. Evansi* -	20-30 μ by 1-2 μ
5.	*T. equinum* -	20-25 μ by 2-3 μ
6.	*T. Gambiense* -	18-25 μ by 2-2·8 μ
7.	*T. Ugandense* -	18-26 μ by 2-2·5 μ
8.	*T. Theileri* -	30-65 μ by 2-4 μ
9.	*T. Transvaliense* -	18-50 μ by 4-6 μ

Considerable variation exists between the data of observers, and though these figures can be considered as approximately correct, they do not suffice for distinguishing the various species.

Whether it will be possible to distinguish nearly allied species morphologically, *e.g.*, *T. Brucei* and *T. Evansi*, remains to be seen. Differences in the position of the centrosome and differences in staining properties hardly suffice in similar species that resemble one another closely, and at present the only certain method is their pathogenic properties.

Multiplication of trypanosomata takes place by longitudinal division, the nucleus and the centrosome divide into two or more parts. The trypanosome becomes more or less quadrangular in form, and from each centrosome a new flagellum is seen starting. Other modes of multiplication are described—conjugation, transverse division, formation of amoeboid forms, etc. Sexual differentiation has also been suspected, anyhow, it is certain that in the organs of a case of

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6.	*T. Gambiense*	-	18-25 μ by 2-2·8 μ
7.	*T. Ugandense*	-	18-26 μ by 2-2·5 μ
8.	*T. Theileri*	-	30-65 μ by 2-4 μ
9.	*T. Transvaliense*	-	18-50 μ by 4-6 μ

Considerable variation exists between the data of observers, and though these figures can be considered as approximately correct, they do not suffice for distinguishing the various species.

Whether it will be possible to distinguish nearly allied species morphologically, *e g.*, *T. Brucei* and *T. Evansi*, remains to be seen. Differences in the position of the centrosome and differences in staining properties hardly suffice in similar species that resemble one another closely, and at present the only certain method is their pathogenic properties.

Multiplication of trypanosomata takes place by longitudinal division, the nucleus and the centrosome divide into two or more parts. The trypanosome becomes more or less quadrangular in form, and from each centrosome a new flagellum is seen starting. Other modes of multiplication are described—conjugation, transverse division, formation of amoeboid forms, etc. Sexual differentiation has also been suspected, anyhow, it is certain that in the organs of a case of

trypanosoma infection, numerous strange forms can be seen, of which nothing is as yet definitely known.

Inoculation.—The most certain and rapid method is intraperitoneal, *e.g.*, in the case of *T. Lewisii*, but subcutaneous is almost equally certain, and in *T. Brucei* scratch inoculations nearly always succeed.

Blood Examination.—If present in fair quantity there is no difficulty in detecting them fresh with a low power. If very scanty, it may be necessary to centrifugalize the blood. The most delicate test of a successful infection which may have resulted, even though no parasites be found, is a subinoculation into a highly susceptible animal.

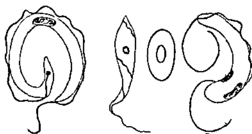


Fig. 88. *T. Soleae, T. Avium* (*after* DANILEWSKY) .*Tp. Borreli* (*after* LAVERAN)

Where parasites cannot be found by an ordinary examination in the blood, they may, however, be readily discovered in the oedematous swellings so often found in trypanosomiasis. Thus it is often extremely difficult, if not impossible, to detect trypanosomes in the blood of a rabbit infected with *T. Brucei*, yet they are easily found in the oedematous fluid about the ears, muzzle, etc.

Genus Trypanoplasma

1. *Trypanoplasma Borreli.*—Twenty μ long, three to four μ broad. Each flagellum, fifteen μ long. It is curved in shape. The undulating membrane on the convexity. The anterior end is more pointed than the posterior. Found in the blood of the red eye (*Leuciscus erythrophthalmus*).

2. *Trypanoplasma Danilewski.*—Fifteen to twenty μ long, less than one μ broad. Found in the gut of leeches (possibly derived from the blood of some animal). (Fig. 79).

Chapter XXVIII

FILARIA

The *Filariidae* form one of the families into which the *Nematodes* are divided. Other families in this sub-order are the *Ascaridae*, *Strongylidae*, *Anguillulidae*, etc. The *Filariidae* are divided into several genera, only one of which concerns us immediately, viz., the *Filaria*, and first, we shall consider those species of filaria which have their embryos in human blood. They are the following:

1. *F. Bancrofti*, syn, *F. nocturna* (MANSON).
2. *F. diurna.*
3. *F. Perstans.*
4. *F. Ozzardi.*
5. *F. Demarquai.*
6. *F. loa.*
7. *F. Megalhaesi* (adults).
8. *F. Gigas* (PROUT).

The following are the characters of the embryos of each species:—

F. bancrofti.—Occurs at night in the peripheral blood, found in the internal organs by day, especially in the vessels of the lungs. *Ce. Argyrotarsis* and *Ce. Albipes* are efficient hosts.

1. Length about three hundred μ fresh (one hundred and eighty μ, stained specimens) by seven to eleven μ wide.

2. Enclosed in a sheath considerably longer than body of embryo.

3. If placed rapidly beneath microscope shews at first active progressive movement (ANNETT and DUTTON), later the anterior tip of the sheath appears to become attached to the glass, and movements of the embryo, though active, are not progressive.

4. The embryo shews an anterior abruptly rounded off end and a posterior, tapering for two-fifths the length. There is a six-tipped prepuce and a short very fine fang.

5. The stained specimen shews (i) an irregular transverse break about twenty-one per cent. of the length.

(ii) A V-shaped spot or transverse irregular break at a distance of about thirty per cent. of the length from anterior end. Nearly always present.

(iii) An area of varying length with cells loosely arranged, sixty-three per cent. length. This is constant and represents the central aggregation of fresh specimens.

(iv) An irregular, sometimes oval spot, often present, eighty-five per cent. length.

(v) A small central bright spot occasionally, ninety-one per cent. length.

F. diurna.- No differences are distinguishable between the embryos of *F. diurna* and *F. nocturna* either in the fresh or stained specimen (ANNETT and DUTTON). DUTTON and ANNETT have found the embryos taken from the adult female *F. loa* to be practically identical with those of *F. diurna*, and describe a case in which infection with *F. loa* was associated with embryos present in the blood during the day and not to the same extent at night.

Embryos of *F. loa*, taken from the female, are described by ANNETT and DUTTON.

1. Length, 208 μ
2. Possess a sheath.
3. Spots as follows : —

(i) An oval or diamond-shaped spot, twenty-four per cent. length from anterior end.

(ii) An indistinct lateral area containing scattered nuclei, thirty-seven per cent. length.

(iii) A longer portion of worm which stains badly, sometimes divided into anterior and posterior portions.

(iv) A small lateral bay, eighty-six per cent. length.

F. perstans.—Embryos present in peripheral blood day and night.

1. Length, two hundred μ by four μ to five μ breadth in fresh, and about ninety μ in stained preparations.
2. Do not possess a sheath.
3. Movements extremely active, and progressive movement continues for many hours. They possess the power of considerable elongation and shortening.
4. No hooked prepuce, a fang is generally observed protruded and retracted. The body tapers gradually for two-thirds of length, and is abruptly truncated at the tail and slightly bulbous.
5. In stained specimens the following spots are made out :—

(i) A narrow irregular transverse band at distance of 26·4 per cent. length, nearly always present.

(*ii*) A *wider irregular transverse spot*, at thirty-six per cent. length. Occasionally.

(iii) The largest of the spots an irregular transverse area, sixty-three per cent. length. Not always present.

(iv) A very inconstant central bright speck at eighty-three per cent. length.



Fig. 89. *F. Bancrofti, Embryo shewing sheath*; *F. Perstans, Embryo (sheathless)*; *F. Bancrofti (Embryo), prepuce and fang (above)*; *F. Perstans (Embryo), fang (below)*

6. They are readily distinguished from the embryos of *F. Ozzardi* by their blunt tails.

7. The observations of FIRKET and CHRISTY point to the fact that there is more than one species of *F. Perstans*.

F. Ozzardi:—

1. Length, one hundred and seventy-three to two hundred and forty μ by four to five μ.

2. They are *sharp-tailed* and sheathless.
3. They have no periodicity.

F. Demarquaii :—

1. Length, two hundred and five μ by five μ.
2. Tail sharp and sheathless.
3. Cephalic armature, ill-developed prepuce and spine.
4. A V spot exists fifty-two μ from the head (seen in wet films).
5. There is no periodicity.
6. *Ce. Argyrotarsis* and *Ce. Albipes* are inefficient hosts.

F. loa.—Two cases of infection with the adult *F. loa* have been described in which *F. diurna* occurred in the blood. It is possible then that *F. diurna* is the embryonic form of *F. loa* (*vide F. diurna* antea). On the other hand, *F. diurna* embryos are indistinguishable from those of *F. Bancrofti*, the adult forms of which are well known.

F. Megalhaesi.—Adults only known.

F. Gigas :—

1. Blunt tailed.
2. Has no sheath.

The Characters of the Genus Filaria

They are long slender worms of almost uniform breadth throughout their length. The anterior extremity is rounded, and the mouth often has no lips. The males are distinctly smaller than the females. They have an incurved or spiral tail, the latter sometimes having lateral membranous outgrowths. They usually have

four pre-anal and a variable number of post-anal papillae and spicules, which vary in size and appearance. In the females the vulva opens in the neighbourhood of the mouth. The host in which the filaria reaches full maturity, giving rise to embryos, is the definitive host, the other host is the intermediary or secondary host. Thus *F. Bancrofti* has for its definitive host, man, for its intermediary host, certain species of *Culicidae*.

F. recondita.—Definitive host, dogs. Intermediary host, *P. serraticeps* (dog-flea).

Adult Filariae

1. *F. Bancrofti.*—The adult male and females are found together, sometimes in the lymphatics or in cyst-like dilatations of these. The embryos gain access to the circulation by the thoracic duct.

2. *F. diurna* —Adult form doubtful. According to Annett and Dutton it is *F. loa*.

3. *F. Perstans.*—The adults were found by Daniels at the root of the mesentery, behind the abdominal aorta, and beneath the pericardium.

4. *F. Ozzardi.*—Adults found by Daniels in the sub-peritoneal tissue.

5. *F. Demarquaii.* —Adults doubtful. A female form has been described differing slightly from that of *F. Ozzardi.*

6. *F. loa.*—Adults found in the subcutaneous areolar tissue, also in the eyelids, and beneath the conjunctiva.

7. *F. Megalhaesi.*—Adults only known, found in the left ventricle of the heart by Figueira de Saboia.

8. *F. Gigas.* -Adults unknown.

The following are the characters of the respective species :—

F. Bancrofti.—Males and females found together in lymphatics.

♀ 1. Length, eighty-five to one hundred and fifty *mm*.

2. Distinct neck; one-third width of body.

3. Body plain, tapering somewhat abruptly to neck, and tapering towards tail.

4. Cuticle with striations.

5. Tail ends bluntly, and has a small depression, surrounded by two small lips.

6. Mouth simple, minute, terminal.

7. Ova twenty-five to thirty-eight μ by fifteen μ.

8. Anus ventral opening on summit of a bilobed papilla.

♂ 1. Length, eighty mm.

2. Body cylindrical, tapering to tail. No neck.

3. Mouth circular, simple, terminal.

4. Cloaca ventral, four pairs pre-anal, four post-anal, papillae (MANSON doubts the presence of these). Two unequal spicules.

5. Genital tube simple. Oesophagus thick-walled.

6. Tail vine-tendril like, with one or two spirals.

F. Perstans :

♀ 1. Length, seventy to eighty mm.

2. Neck longer than *F. Bancrofti.*

3. Body without markings.

4. Tail incurvated. Tip of tail mitred. This is *characteristic of this species.*

5. Mouth minute, simple.

6. Embryos in utero, blunt tailed, not sheathed.

♂ 1. Length, forty-five mm.

2. Head end as in female.

3. Two caudal ends much coiled.

4. One spicule and two papillae.

5. Low describes four pairs of pre-anal and one pair of post-anal, minute papillae.

F. Ozzardi.—Adult.

1. Dimensions much the same as those of *F. Bancrofti.*

2. Distinguished by the *bulbous* tail; in *F. Bancrofti* it is not bulbous but circular.

F. loa.—Adult forms travel about in connective tissue.

♀ 1. Length, thirty to forty mm. (average). Varies from sixteen to seventy mm. Breadth, 0·57 mm.

2. No neck. Head cone shaped.

3. Body cylindrical, tapers sharply towards head and tail.

4. Cuticle with bosses, except over the head.

5. Tail terminates in a short incurved portion, and has two small tubercles at its extremity.

6. Mouth simple.

7. Ova, containing embryos, thirty-five μ by twenty-five μ.

8. Anal orifice on low, broad papillae, 0·3 mm. from the tip.

♂ 1. Length, twenty-five to thirty mm.

2. Uniform thickness except at head and tail.

3. Cuticle with bosses, but not so numerous as in the female.

4. Tail not spirally twisted, merely incurved. It possesses well-marked lateral alae.

5. Three well-marked pre-anal papillae and two unequal post-anal papillae. Two slender unequal spicules.

F. Megalhaesi.—Adult males and females in left ventricle.

♀ 1. Length, one hundred and fifty-five *mm.* by 0·7 mm.

2. Club-shaped oral end.

3. Swollen oesophagus well marked.

4. Mouth simple.

5. Cuticle, fine striations.

♂ 1. Length, eighty-three mm. by 0·4 mm.

2. Four pairs pre-anal, four post-anal papillae, and two spicules.

To Examine Blood for Filaria Embryos

The technique varies somewhat according to what end the observer has in view.

1. To facilitate detection, it is well, as Manson advises, to make thick films of blood. Dry. Then wash out the haemoglobin with water or one-third per cent. acetic acid, and stain with haematin, or gentian violet, or fuschin.

For the latter stains, a few drops of a saturated alcoholic solution of the dye are added to half a watch-glass full of water.

In these methods no fixation has taken place, and the parasites are exposed directly to the action of a watery die.

2. (For studying the minute structure of the embryos, the above method is not advisable). Make a film in the ordinary way. Fix in alcohol and stain with haematin. The 'spots' and granules of the embryo are most beautifully shewn.

FILARIA IN MAMMALS

F. immitis.—Adults found in right ventricle of dog, fox, and wolf. Embryos in blood. Embryos develop in the malpighian tubes of *Anopheles*. Afterwards they enter the general body cavity and pass towards the labium

Dogs in the tropics commonly harbour this filaria. The filariae are most numerous at night.

F. recondita.—A single female adult has been found in kidney of dog.

Embryos in Blood.—Embryos develop in *P. serraticeps* (dog-flea), and *P. irritans* (man and dog); also in a dog tick. They are found in the intestine and body cavity. The filaria has, however, not been transmitted from infected fleas to healthy dogs.

F. equina.—Serous cavities, intestines, and liver of horses, donkeys, and mules.

Embryos in Blood.—Appear like *F. Bancrofti*, but smaller.

F. haemorrhagica.—Male and female live together in tissues of horse and donkey. They form hemispherical tumours the size of a nut beneath the skin. These burst and discharge blood. Fresh tumours appear in from one to two days.

F. irritans.—Found in 'summer sores' of horses and donkeys.

F. Evansi.—Lung and mesentery of camel. Embryos in blood.

F. lacrymalis and *F. palpebralis.*—About the eyes of horses and cattle.

F. osleri.—The adults cause broncho-pneumonia in dogs.

5. Three well-marked pre-anal papillae and two unequal post-anal papillae. Two slender unequal spicules.

F. Megalhaesi.—Adult males and females in left ventricle.

♀ 1. Length, one hundred and fifty-five mm. by 0·7 mm.

2. Club-shaped oral end.

3. Swollen oesophagus well marked.

4. Mouth simple.

5. Cuticle, fine striations.

♂ 1. Length, eighty-three mm. by 0·4 mm.

2. Four pairs pre-anal, four post-anal papillae, and two spicules.

To Examine Blood for Filaria Embryos

The technique varies somewhat according to what end the observer has in view.

1. To facilitate detection, it is well, as Manson advises, to make thick films of blood. Dry. Then wash out the haemoglobin with water or one-third per cent. acetic acid, and stain with haematin, or gentian violet, or fuschin.

For the latter stains, a few drops of a saturated alcoholic solution of the dye are added to half a watch-glass full of water.

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2. (For studying the minute structure of the embryos, the above method is not advisable). Make a film in the ordinary way. Fix in alcohol and stain with haematin. The 'spots' and granules of the embryo are most beautifully shewn.

Avian Filariae

Filarial embryos are very common in the blood of birds, and the adult forms are found in the most diverse positions, notably in the subcutaneous tissues. In some form the embryos appear to be confined to the lymph.

In the description of *Avian filaria* the following should be noted:—

1. The species of bird concerned.
2. The site of the adult filariae.
3. The description of the adult filariae, female and male; the use of Cobb's formula gives uniformity to descriptions. The measurements are taken with the animal in profile from the anterior end.
    (i) To the base of the oesophagus.
    (ii) To the nerve ring.
    (iii) To the cardiac constriction.
    (iv) To the vulva in the female, or to the middle in male.
    (v) To the anus, noting when this is terminal.

At each of these points transverse measurements are taken and noted below the above, so

Longitudinal.
Transverse.

The unit of measurement is one hundredth part of the length of the worm.

This formula should be used with caution, since it rests on the assumption that the proportions of the various parts of the body are constant in different individuals (Shipley).

Drawings should be made of the head and tail, and the mouth, anus, and vaginal orifice carefully described.

4. The description of the embryo. Where found, blood or lymph. Presence of a sheath. Length and breadth of embryo and sheath. The exact description of spots and the distance of these from the anterior extremity. The following spots and markings may be seen:—

(i) A transverse slit, about twenty-five per cent. length. Sometimes not seen.

(ii) A clear, sometimes lateral, sometimes transverse spot, about thirty to forty per cent. length.

(iii) A long space in which the nuclei are loosely arranged, often ending anteriorly and posteriorly in clear space. About sixty-five per cent. length.

(iv) A small spot, about seventy-six per cent. length.

(v) Very small lateral spot or slit, ninety per cent. length.

Development of Filaria in the Mosquito

Experiments made so far have been chiefly with *F. nocturna* (*F. Bancrofti*).

Both *Anopheles* and *Culex* mosquitoes may act as the hosts of *F. Bancrofti*. Certain species of both genera, however, do not act as hosts. The following have been shewn to act as hosts:—

C. pipiens	*P. Costalis*
C. ciliaris	*Mym. Rossii*
C. Fatigans	*Myz. Sinensis*

The following have been shewn by BANCROFT not to allow full development to take place. In some species partial development occurs, the larva, however, eventually disappearing:—

C. *notoscriptus* (SKUSE)
C. *annulirostris* „
C. *hispidosus* „
C. *vigilax* „
C. *Nigrothorax* (MACQUART)
C. *procax* (SKUSE)
[A.] *Musivus* „

GRASSI and NOE'S experiments shew that *F. immitis* is capable of developing in *A. claviger*. As regards the re-infection of a healthy dog, the experiments are somewhat inconclusive, for, in the dog used, a single immature worm only was found, about sixteen days after the period of 'biting.' They state, however, that of a batch of *Anopheles* dissected before the 'biting,' many of the labia contained filaria, whereas, of a batch dissected after the 'biting,' none contained filaria, the conclusion being that the filaria had escaped through the labia into the blood of the dog during the 'biting.'

Seven stages of development of the embryo are usually described. The following is a resumé of the changes undergone in *Culex pipiens*:—

First Stage.—One hour after removal of blood by mosquito, the sheath is cast and the embryos exhibit active locomotive movements. In twelve to eighteen hours many have bored through the stomach wall, and have reached the

muscles. Some die within the stomach. In the muscles the cuticular striation disappears, movement ceases, and the body becomes thicker.

Second Stage.—For two to three days the embryo becomes much thicker, and the mouth begins to be faintly indicated.

Third Stage.—An anus appears in front of the tail, and a mouth is very distinct with four fleshy lips. Cells are seen in the body, and these form an alimentary and tegumentary layer. The embryo is now about 0·3 mm. long.

Fourth Stage.—Rapid growth takes place, and the tail becomes relatively smaller.

Fifth Stage.—Lengthening takes place. The whole worm becomes fibrous and transparent in appearance. It has cast the cuticle. Some large cells at the end of the tail form papillae which are characteristic of this stage of the larva. The parasite is now about 1·5 mm (one-sixteenth inch). Time, about seventh day.

Sixth and Seventh Stages.—Movements become more active and, when the filariae have reached their highest stage of development in the thoracic muscles, they leave that tissue and travel forward in the direction of the head of the mosquito (Low and James). They reach the loose tissue about the salivary glands and pass into the neck. Some are found in the abdomen. Numbers of the filaria larvae enter the lower part of the head, lying beneath the large head ganglia. Eventually one or more worms pass into the substance of the labium, where they are readily found by dissection. The larva at this stage measures about one-sixteenth inch in length.

According to DUTTON, who has very minutely described the structure of the proboscis, the worms can only leave the labium at one point, *i.e.*, by perforating an extremely delicate membrane, which closes in the extreme end of the labium (see p. 166). If they escape elsewhere, they must penetrate the dense and hard chitinous envelope of the labium—a very improbable occurrence.

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APPENDIX

APPENDIX

BLOOD-SUCKING FLIES

The Diptera or flies are two-winged insects (the posterior pair of wings are transformed into *halteres*), and are so distinguished for example from the Hemiptera or bugs which generally have four wings. In the Diptera the metamorphosis is complete, eggs, larva, pupa, insect, in the Hemiptera it is not so. The following classes have blood-sucking habits.

The *Nemocera* (νῆμα, thread, κέρας, antenna), including the following families —

1.—*Blepharoceridae*.

Wings iridescent, no 'discal' cell on wing (the discal cell lies between the second posterior cell and the second basal cell). They resemble midges. The larvae have suckers, and are found attached to stones in the water.

Genus *Curupira*.

2.—*Culicidae*. Mosquitoes or gnats.

3.—*Chironomidae*. (Midges.)

[Genus *Chironomus*] not blood-sucking. Larvae are blood worms, 'Vers de vase.'

Genus *Ceratopogon*. Very minute midges. Wings generally spotted. Larvae mostly non-aquatic.

C. varius. A pest in Scotland.

4.—*Psychodidae*. (Moth flies.)

Very small. Antennae very hairy. Wings very hairy. Larvae of some genera amphibious.

Genus *Phlebotomus*. S. Europe, etc.

5.—*Simulidae* (Sandflies, Buffalo gnats.)

Small hump-backed flies. Antennae destitute of hairs. Wings relatively large. Proboscis short, thick, consisting of epipharynx and hypopharynx. Antenna eleven segments. Palpi four segments. Larvae aquatic.

Genus *Simulium*.

The *Brachycera* (βραχύς short, κέρας antenna) include the following :—

1.—*Tabanidae* (Horse-flies or gad (=sting) flies).

Large flies. Antenna three-jointed, not terminating in a style or arista (the arista (when bristle-like) or style (when thick) being an appendage of the terminal portion (flagellum) of the antenna). Labium enclosing four stylets in ♂, six in ♀. The terminal joint of the palpi is inflated, and the palpi hang down in front of the proboscis.

(*a*) Genus *Tabanus*.

Proboscis short and thick, vertical in the female, oblique in the male, terminal joint of antenna crescentic. Flight 'humming.' Attacks horses, cattle, man.

(*b*) Genus *Haematapota*.

Terminal joint of antenna not crescentic. Wings adjacent, like the sides of a roof. No ocelli. Flight silent.

H. pluvialis. Common in woody lanes in England in the summer.

(*c*) Genus *Pangonia*.

Proboscis often long, thin, horizontal. In some, three to four times length of body, piercing, even when the fly is on the wing. Accessory eyes (ocelli) generally present. Hind tibiae spurred. The 'Seroot' fly of Nubia is probably a species of Pangonia. Whether the 'Zimb' fly of the Arabs or the 'Tsaltsallya' is the same is doubtful.

(*d*) Genus *Chrysops*.

Three ocelli Second joint of antenna as long as first. Eyes golden green Flight silent. Wings widely separated. Spotted. Hind tibiae spurred.

Ch. caecutiens attacks the eyes especially.

(*e*) Genus *Hadrus*.

H. lepidotus = Motuca fly of Brazil.

The third group (*Cyclorrhapha Schizophora*) include the muscinae, sarcophagidae, and oestridae. The last two groups are not blood-suckers, but are included here for their pathological interest; also only some of the first group are blood-sucking.

1.—*Muscinae*.

Antennae have three joints and an arista, wing veins less complex than in *Brachycera*.

(*a*) Genus *Musca*. House flies (not blood-suckers).

(b) Genus *Calliphora.* Blow flies or blue bottles (not blood-suckers).

(c) Genus *Lucilia* Green bottles

L. macellaria. The larva of this fly is the American 'screw worm,' infesting the nasal fossae and frontal sinuses of man.

(d) Genus *Stomoxys*

Proboscis horizontal, long and firm Third segment of antenna three times as long as second Arista feathered on its upper surface only Palpi not longer than the epistome

S. Calcitrans resembles the house-fly, but its head is raised. Attacks cattle, occasionally man Attempts to transmit *T. Brucei* with this have been negative

(e) Genus *Haematobia*

Small flies. Palpi as long as proboscis, arista feathered above and partly below Attacks cattle fiercely

(f) Genus *Glossina.*

Arista feathered above. *Vide antea*

2.—*(Sarcophagidae).*

Not blood-sucking Arista feathers at the base, bare at the tip. Large flies, about 14 millimetres long

Genus *Sarcophaga* Elongated thorax, three black bands, abdomen spotted. Third segment of antenna three times the second segment

S. carnaria, S. magnifica and S. Ruficornis (India), give rise to terrible forms of myiasis in man and animals

3.—*(Oestridae)* (Bot. (=Larva) Flies).

Not blood-sucking Large flies. Proboscis rudimentary. Antenna very short. Arista segmented. Flight humming.

(a) Genus *Gastrophilus, e.g., G. equi.* The white eggs can be easily seen on the horse's hair The larvae are swallowed and they attach themselves to the mucosa of the stomach

(b) Genus *Hypoderma, e.g., H. lineata* Larvae produce ox warbles (=tumours) in the ox.

(c) Genus *Oestrus, e.g., O. Ovis* Larvae in the respiratory passages of the sheep.

(d) Genus *Cephalomyia e.g., C maculata* In the camel.

(e) Genus *Cephenomyia, e.g. C rufibarbis.* In red deer. Scotland.

(f) Genus *Dermatobia, e.g., D. cyaniventris.* Larva is the 'ver macaque' (America), producing myiasis in man and cattle.

(*g*) Genus *Ochromyia*, *e.g.*, *O. anthropophaga*. Larva is the 'ver de Cayor' (Senegal), producing myiasis in man.

Myiasis is common in Africa and in the tropics, but the larvae have been identified in but few instances as yet.

The *fourth group*, the *Pupipara* (*to which Glossina also* belongs, from the point of view of its life history), comprises:

1.—*Hippoboscidae.*

Labium absent. Proboscis consisting of epipharynx and hypopharynx ensheathed by the maxillae. Palpi wanting. Deposit larvae which subsequently became pupae. They suck the blood of mammals and birds. Their wings are often very minute.

(*a*) Genus *Hippobosca* (spider flies).

Wings large, obtuse. No ocelli; arista nude; legs long and extended.

H equini. Runs rapidly over the body; is the [New] forest fly of England.

H. camelina. Attacks camels in Egypt.

H. rufipes transmits Trypanosoma theileri.

(*b*) Genus *Melophagus.*

Wings extremely minute. No arista on antennae.

M. ovi is the sheep 'tick.' Four millimetres long.

(*c*) Genus *Ornithomyia.*

Wings large. Four millimetres long.

O. avicularia Occurs on birds.

(*d*) Genus *Lipoptera*, *e.g.*, *L. cervi* on the red deer.

(*e*) Genus *Stenopteryx*, *e.g.*, *S. hirundinis* of the swallow.

2.—*Nycteribiidae.*

Found on bats. They have no wings.

FLEAS †

Fleas, or Aphaniptera, are considered to be aberrant forms of flies, and hence follow naturally after the division Pupipara of the flies

† Rabinowitsch has obtained positive results in the transmission of the rat trypanosome by fleas. In this case, and in the case of Glossina there is no evidence to show that the trypanosome undergoes any developmental change; nor, even in the case of Glossina, has it actually been shewn that trypanosomes occur in or on the proboscis during the biting of an uninfected animal.

LIFE HISTORY

Eggs: About a dozen are laid, in floors, in cracks, etc.; sometimes in the hair or fur of animals. They are 0·7 by 0·4 millimetres (*P. irritans*). The eggs hatch in about a week or more.

Larvae: Are worm-like, whitish, consisting of fourteen segments. They are about one-and-a-half by one-tenth millimetres in size. They feed on organic refuse (?) on blood. In about eleven days they are full grown. They wrap themselves up in a cocoon, moult, and become

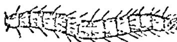


Fig. 90. Larva of a Flea (P. [illegible]) x 20
(After RAILLIET)

Nymphs.—After the lapse of another eleven days the flea emerges (the evolution thus taking about a month).

Adults.—The female has a concave back, the male a convex back.

ANATOMY

1. The head is small, not distinctly separated from the body.

2. The antennae are placed in fossae behind the eyes. They consist of two basal segments, and a third of diverse form irregularly segmented. The maxillary palpi must not be mistaken for them.

3. The mouth consists of (*a*) epipharynx (the central stylet) serrated above, tubular below, (*b*) two serrated mandibles hollowed on their inner surfaces and forming with (*a*) a gutter, along which the blood flows; (*c*) a labium single for a short distance, then bifurcating and forming two labial palps, which form a sheath for the piercing organs (*a*) and (*b*), (*d*) two maxillae having the form of expanded plates, each bearing a four-jointed palpus.

4. The thoracic segments, three in number, are separate.

5. Abdomen consisting of nine segments, overlapping, the dorsal and ventral portions not being united, and so allowing of distension.

6. Spines, arranged in comb-like fashion, exist on the lower part of the head, pro- and meta-thorax, and abdomen. These are of great importance in classification.

The *Aphaniptera* comprise (1) the *Pulicinae* (fleas proper), (2) the *Sarcopsyllinae.*

The *Pulicinae* have—

1. Small head.
2. Labial palps four joints.
3. They are never stationary parasites.

There are three genera: (1) *Pulex*, (2) *Hystrichopsylla*, (3) *Typhlopsylla.*

Genus *Pulex.*

1. Well-developed eyes.
2. The eggs are not fixed to hairs, etc.

P irritans.

1. No spines on lower portion of head.
2. No spines on pro-thorax.
3. Third joint of antennae incised.
4. Parasitic on man.



Fig 91 *P. irritans*, x 20. (*After* RAILLIET)

P serraticeps.

1. A comb of seven to nine spines on each side of the head.
2. Pro-thorax dorsally; a similar comb.
3. Parasitic on dogs (? same species on cats).

P. goniocephalus.

1. Head bent into an obtuse angle. Above and laterally has combs of five to six spines.
2. Pro-thorax, posteriorly, six long narrow spines.
3. Parasitic on rabbits.

P. avium.

1. Head no spines
2. Prothorax, twelve to thirteen posteriorly.
3. Parasitic on birds. Doubtful if all are of the same species

There are a large number of other species

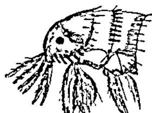


Fig. 92. *P Serraticeps*, × 30 (*After* RAILLIET)

Genus *Hystrichopsylla*

1. No eyes.
2. Head truncated.

A single species exist, on the mole and vole

Genus *Typhlopsylla.*

1. Eyes rudimentary or absent
2. Head elongated, rounded in front.

The species are distinguished by the number of their spine combs, *e.g.*, *T. octatenus*, *hexactenus*, *pentactenus*, etc. They are parasitic on bats, moles, shrews, mice, rats, voles, field-mice.

The *Sarcopsyllinae*

1. Head large
2. Thorax narrow
3. The terminal joint of the antenna is not segmented

Comprise three genera (1) *Sarcopsylla*, (2) *Rhyncopsylla*, (3) *Vermipsylla*.

Genus *Sarcopsylla*

1. Head angular.
2. Eyes small
3. Maxillae small

4. Epipharynx elongated.
5. The abdomen in the female capable of great distension They imbed themselves in the tissues.

S. penetrans (CHIGGER). On man, pig, horse, goats, and, even it is said, birds.

S. gallinarum. On the eylids and necks of fowls (Ceylon).

Genus *Rhyncopsylla.*

1. Head rounded.
2. Maxillae curved.

A single species found on parrots and bats. Are fixed like ticks, with the body free.

Genus *Vermipsylla.*

1. Head rounded.
2. Maxillae large and triangular.

V. Alikurt. Parasitic on horses (Turkestan). Fixed with the body free.

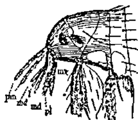


Fig 93. P. Goniocephalus, x 30. (*After* RAILLIET)

LITERATURE

Cambridge Natural History. *Insects*, Pt. II.
Railliet. *Traité de Zoologie medicale et agricole.*

STAINS, NUMERICAL DATA, ETC.

FIXING AND HARDENING SOLUTIONS

Alcohol is a fixative and dehydrating medium, and for ordinary work is the most convenient. The tissues, in small pieces, may be placed directly in methylated spirit (ninety per cent. alcohol), or absolute alcohol (ninety-eight per cent. alcohol). Change the alcohol a few times. Or pass through

fifty, seventy-five, ninety-five, one hundred per cent. alcohols, leaving a few hours in each. After hardening, if the specimens *are not to be imbedded immediately, transfer to alcohol of* about eighty per cent. for preserving. After the use of other fixatives, specimens should be washed and transferred to eighty per cent. of alcohol for preservation

Rectified spirit of the British Pharmacopaeia, is equal to *eighty-four per cent. alcohol*

Methylated spirit, containing *wood naptha*, is equal to ninety per cent. alcohol.

Ordinary methylated spirit contains mineral naphtha, and should not be used

Absolute alcohol is equal to ninety-eight per cent alcohol For practical purposes the dilution of alcohols is sufficiently accurately made by means of the diluting formula (p. xvii)

Zenker's Fluid:—

Potassium Bichromate - -	2 5 grammes
Sodium Sulphate - -	1 0 grammes
Corrosive Sublimate-	5 0 grammes
Water - - -	100 0 grammes

Add glacial acetic acid to this stock solution, in the proportion of five grammes to one hundred c c, before use. Fixation is complete in one to twenty four hours. *Wash thoroughly* in alcohol to which enough iodine has been added to give a dark-brown solution. Or, if alcohol is undesirable use tincture of iodine, two parts, potassium iodide, one part, glycerine, fifty parts, water, fifty parts. Renew until no further decolouration takes place

Haematin or eosin and methylene blue give good results for malarial tissues

Orth's Fluid.—This is Muller's fluid to which formalin (*i.e.*, formaldehyde forty per cent solution) is added in the proportion of ten c c to one hundred c c of Muller before use

Fixation of small pieces takes place in two to three hours, if kept warm. Wash thoroughly. Pass through alcohol

Muller's Fluid.—Potassium Bichromate, 2½ parts
Sodium Sulphate 1 part
Water - 100 parts

Add a *little camphor or naphthalin to prevent the growth* of moulds. Change the fluid after twenty-four hours, and then every few days for the first week. Tissues are ready in a fortnight or three weeks. They may be left much longer. Fix

in the dark Wash thoroughly in water till colourless. Transfer to alcohol, seventy to eighty per cent., for preservation.

Flemming's Solution.—Chromic acid, 1 per cent., 15 vols
Osmic acid, 2 per cent., 4 vols.
Glacial acetic acid - 1 vol.

Mix in the above proportions before use. Use very small pieces. Fixation is complete in about twenty-four hours. Blackening due to the osmic may be removed by hydrogen peroxide. *Blood films may be fixed in this solution.*

Tissues thus fixed may be preserved in equal parts of alcohol and glycerine.

Formalin (forty per cent. solution of formaldehyde).—Use two to five per cent solution in water. Small pieces are fixed in twelve to twenty-four hours. They may be left in solution or transferred to alcohol.

Corrosive Sublimate.—Best used as a concentrated alcoholic solution (or aqueous may be used). Fixation takes place in a few hours. Wash thoroughly in water and transfer to iodine solution (*vide* Zenker's fluid) till iodine no longer decolourized.

The concentrated alcoholic solution is a most rapid fixing and hardening reagent, and sections can be cut in a very short time, if small pieces are used.

Decalcifying Solution.—Tissues require fixing before and after these solutions—

(i) Phloroglucin, one gramme, nitric acid, ten c.c, water, one hundred c c., *or*

(ii) One to five per cent. solution of nitric acid in water or alcohol. Change the fluid daily. Decalcification takes place in two to three days.

(iii) Picric acid, a saturated solution (= about 0·75 per cent.) containing crystals. Decalcification may take weeks or months *Wash in alcohol.*

Eau de Javelle (dissociating and decolourizing solution).—Add to a concentrated aqueous solution of chloride of lime a solution of potassium oxalate as long as a precipitate is formed. Filter and dilute if necessary. This may be used for softening the chitinous skeleton of mosquitoes and for decolourizing Madura fungus, etc.

For Fixing Paraffin Sections to the Slide

1. Celloidin, one part, oil of cloves, two parts; *or*
2. Thin solution of white shellac in creosote; *or*

3. Thoroughly mix equal parts of white of egg and glycerine; filter. This is one of the simplest and best means; *or*

4. Filtered white of egg, 50 c.c.
   Glycerine - - 50 c.c.
   Sodium salicylate - 1 gramme

Shake well and filter (this takes about a week). The solution keeps well for six months or more; *or*

5. Simply use fresh white of egg, smear thinly over, dry.

Mounting Media, etc.

1. *Farrant's Solution.*—

i. Take equal parts of glycerine and a saturated solution of arsenious acid. Add powdered gum arabic till the solution is saturated; *or*

ii. Pure gum arabic, 40 grammes
   Water - - 40 c.c.
   Glycerine - - 20 c.c.
   Carbolic acid - 1 gramme
   [or Thymol - 0.3 grammes.]

Powder the gum and dissolve in about one hundred and fifty c.c. of water by boiling, add the carbolic acid, dissolved in a a little water, filter through a hot filter, changing when clogged, evaporate until it is about eighty c.c., then add the glycerine.

2. *Acetate of Potassium.*—Saturated solution. Cement the cover-glass with gold size, dammar lac. This is used for osmic preparations, and for glycogen stained with iodine, etc.

Glycerine Jelly.—Glycerine 70 c.c.
   Water - 60 c.c.
   Gelatine - - 10 grammes
   Thymol 0.5 grammes

or one gramme of phenol to each one hundred grammes of the mixture.

Dissolve the gelatine at forty degrees in a water bath, add the glycerine (warmed), powder the thymol and mix with a little water, and stir in, add the beaten up white of an egg, stir continuously, warm to eighty-five degrees, filter through a hot filter.

To Mount Delicate Objects

Place in ten per cent. glycerine, allow this to concentrate in the air, and then transfer to the jelly.

To mount in Jelly.—Remove as much water as possible; place the slide, coverglass, and jelly in the incubator (if necessary). Before cementing see that the layer of jelly is not too thick. If too thick press some out and scrape away; then cool.

Dammar Lac.—Dissolve in equal parts of benzene and oil of turpentine. It does not render preparations as translucent as Canada balsam.

Fixing Blood

As we have already stated, for practical purposes alcohol is absolutely satisfactory. The following solutions have been used, and may prove useful occasionally:—

1. *Osmic acid*.—Osmic acid, 1·0; sodium chloride, 0·6; distilled water, 1·0.

A neat and practical method of using this is to moisten a camel's hair brush with the solution, then to touch the blood drop, and to immediately spread the blood out on the slide with the brush. Wash the brush, after use, in alcohol (Kornilowitch).

2. *Chloroform*.—Instead of heat in staining with Ehrlich's triacid. Fix for five minutes in chloroform (neutral to litmus *paper). Stain for five minutes or more after fixing (Josué).*

3. *Strong Flemming*. Especially for nuclear structures of parasites.

4. *Heat*.—Heat up to one hundred and fifteen to one hundred and twenty degrees C. in a hot oven, and then, when this temperature is reached, allow to cool again in the oven.

5. Osmic acid, two per cent., glacial acetic acid, equal parts. *Expose to the vapour. For delicate work indispensable.*

Staining Solutions for Blood, etc.

1. *Romanowsky stain* (p. 10).
2. *Haematin* (p. 50).—If the solution has become reddish on keeping neutralize with a little ammonia.
3. *Eosin and Methylene Blue* (consecutively).—(*a*) Stain for one to five minutes in a one-half per cent. solution of eosin in sixty per cent. alcohol, wash, dry with blotting paper, and stain (*b*) in a saturated watery solution of methylene blue for thirty seconds to one minute.

This is a useful and simple method for studying the acidophil and basophil reactions of granules.

4. *Safranin*.—(*a*) Safranin, O. (' soluble in water '). The *solution must be heated up to sixty to eighty degrees C., and* subsequently filtered; (*b*) a saturated alcoholic solution of safranin ' soluble in alcohol ' Take (*a*) and (*b*) *partes aequales*. Stain for five to ten minutes or less.

5. *Ehrlich's Triacid*

6. *Ehrlich's Haematoxylin-Eosin*.—Haematoxylin, five grammes; acetic acid, twenty grammes; alcohol, one hundred grammes; glycerine, one hundred grammes; water, one hundred grammes. Allow this to ripen for a month in the sun, then add eosin to the extent of one per cent; stain for twenty-four hours. The solution is best got ready-made.

Solubility of Stains

10 c.c. of saturated alcoholic methylene blue contains 0·068 grammes of the stain

10 c.c. of saturated aqueous methylene blue contains 0·664 grammes of the stain

10 c.c. of saturated alcoholic gentian violet contains 0·442 grammes of the stain

10 c.c. of saturated aqueous gentian violet contains 0·175 grammes of the stain

10 c.c. of saturated alcoholic fuchsin (basic) contains 0·292 grammes of the stain

10 c.c. of saturated aqueous fuchsin (basic) contains 0·066 grammes of the stain (Hewlett)

Löffler's Methylene Blue.—30 c.c. saturated alcoholic methylene blue

100 c.c. of 0·1 per cent caustic potash

Ordinary Methylene Blue.—A saturated alcoholic solution is used as the stock, and a few drops added to a watch glassful of water, according to strength required; or ten per cent. solution is *a convenient strength*

Borax Methylene Blue (Sahli's).—Saturated aqueous solution of methylene blue, twenty-four parts; borax five per cent solution, sixteen parts; water, forty parts

The times necessary for staining are best judged by the *appearance of the films or tissues*

Tissue Stains

1. *Haematin* (p. 50).—Stain for about five minutes, according to the ripeness of the solution. Sections do not readily overstain. Decolourize, if necessary, with one per cent.

alum solution Counterstain, if required, with a weak, watery solution of eosin, one-half to one per cent. ; or the sections may be stained first with a strong eosin solution, five to one hundred per cent., for five to *twenty minutes*. *Combination* of eosin and methylene blue can be used in a similar way.

2. *Alum Carmine.*—Carmine, two grammes ; alum, five grammes ; water, one hundred c.c. Boil together. Filter. Does not overstain.

3. *Ehrlich Biondi.*—Saturated aqueous solution of *rubin s., four parts ; orange g., seven parts ; methylene green*, eight parts. Dilute fifty to one hundred times before using. Stain for twenty-four hours. Wash in alcohol. The sections may, before staining, be treated with acetic acid (two parts in one thousand of water) for a few hours.

Iron Reaction (Haemosiderin) in Malarial Tissues

1. Fix in alcohol.
2. Two per cent aqueous solution of potassium ferrocyanide, five to twenty minutes.
3. Acid alcohol (HCl, one part, seventy per cent. alcohol, one hundred parts), five to ten minutes.
4. Wash in water.
5. Counterstain with alum carmine.

Staining of Amoeba Coli in Tissues (Mallory and Wright)

1. Fix in alcohol.
2. Saturated aqueous solutions of thionin, three to five minutes
3. Two per cent. solution of oxalic acid, one-half to one minute.
4. Wash in water.
5. Dehydrate in alcohol.
6. Clean in oleum origanum cretici.
7. Wash in xyol.
8. Balsam.

Nuclei of the amoebae are brownish red, the nuclei of the 'mastzellen' are blue.

Weights and Measures, etc.

1. Conversion from one temperature scale to another—

$$\frac{C}{5} = \frac{R}{4} = \frac{F-32}{9}$$

Thus to convert 100° F. to centigrade—

$$\frac{100 - 32}{9} = \frac{C}{5} \therefore C = 37{\cdot}7^{\circ}$$

2. *Formula for dilution of Solutions.*—The number of parts required to dilute from one part of a solution of strength *x* per cent. to another strength *y* per cent. is $\frac{x}{y} - 1$

Thus, to dilute a solution from 20 per cent. to 5 per cent., add to each volume of solution $\frac{20}{5} - 1 = 3$ of the diluting fluid.

3. *Approximate Values.*—
   1 cubic centimetre = 17 minims   1 minim = ·059 c.c.
   100 " = 3 ounces, 4 drachms, 20 minims
   1 drachm = 3·55 c.c.
   1000 cubic centimetres = 1·76 pints. 1 fluid ounce = 28·4 c.c.
   4 litres = 7 pints   1 pint = 568 c.c.
   1 gramme = 15 [illegible]ths grains (avoirdupois)   1 grain (avoirdupois) = ·0648 grammes
   1 kilogramme = 2 pounds, 3 ounces, 119·8 grains (avoirdupois).   1 drachm = 1·77 grammes
   5 kilogrammes = 11 pounds   1 oz. = 28·35 grammes
   1 grain apothecaries' = ·0648 grammes
   1 drachm apothecaries' ʒ = 3·88 grammes
   1 ounce apothecaries' ℥ = 31·1 grammes
   15·4 grains apothecaries' = 1 gramme
4. 1 millimetre = ·039 ($\frac{1}{25}$) inches   1 inch = 2·5 centimetres
   1 centimetre = ·39 ($\frac{2}{5}$) inches $\frac{1}{1000}$ inch = ·0253 millimetres.
   1 metre = 3·28 feet $\frac{1}{25}$ inch = 1 millimetre
   $\frac{1}{1000}$ millimetre (μ) = ·0004 inches
   $5\mu = \frac{1}{5000}$ inch $\frac{1}{2}\mu = \frac{1}{50000}$ inches
5. $\pi = 3{\cdot}14159$
   Circumference of a circle $= 2\pi r = \pi d$
   Area of circle $= \pi r^2$

LIST OF APPARATUS

		£	s	d
1.	A Beck microscope, including oil immersion, $\frac{1}{12}$	13	10	0
2.	Glass slides,† 3 x 1, ground edges, per gross ·	0	3	6

† Slides and coverglasses should be kept in spirit or alcohol to prevent their becoming opaque, as only too frequently occurs in the tropics.

		£	s.	d.
3.	Cover glasses, No. 1, ¾-in., ½-oz. - - - -	0	4	0
4	Straight surgical needles, for making blood films and dissecting, ½ doz. (Weiss & Co., Oxford Street) - - - - -	0	1	6
5.	Stoppered jars (4), 13 x 7½ centimetres - -	0	3	6
6.	Porcelain dishes, square, flat, 1 doz. - -	0	1	0
7.	Specimen tubes, flat-bottomed, corked, 3 in. x ¾ in., 50 - - - - - -	0	4	0
8	Slide box to hold 25, sliding, cardboard -	0	1	0
9.	Measures, 100 c.c. and 10 c.c. - - -	0	1	9
10.	Drop-bottle for xylol (a toothpick inserted into the cork of a specimen tube serves the purpose) - - - - - - -	0	0	4
11.	Cedarwood oil bottle (a pin in the cork of a specimen tube makes a convenient dropper for the oil) - - - -	0	0	9
12.	A 'Primus' paraffin burner, for boiling, etc. -	0	12	6

STAINS, ETC.

		£	s.	d.
1.	Romanowsky.—Methylene blue, pure medical, 10 grammes, or in 'soloids' - - -	0	1	8
	Eosin, B A, 10 grammes, or in 'soloids' -	0	1	2
	Sodium carbonate, pure, 1 oz. - - -	0	0	2
2.	Leishmann's Stain—(a) In 'soloids' (Burroughs, Wellcome & Co.) = 0·15 gramme. Dissolve in 10 c.c. of methyl alcohol (or methylated spirit).			
3	Haematin, 5 grammes, - - - - -	0	4	6
	Alum, 1 oz. - - - - - - -	0	0	2
4.	Absolute alcohol, 1 lb. - - - - - -	0	4	0
5.	Xyol, 1 lb. - - - - - - - -	0	2	3
6.	Paraffin wax, melting points, 50 C. and 60 C. 1 lb. 2s. 6d. + 1 lb. 3s.	0	5	6

ADDITIONAL APPARATUS

		£	s.	d.
1.	A mechanical stage, fitting on to *the stage* (not the column) of the microscope - -	3	5	0
2.	Browning's pocket spectroscope, indispensable for urine work in blackwater fever, etc. -	1	0	0

FOR MOSQUITO COLLECTION

	£	s.	d.
1. A lens (the lens of an eye-piece of the microscope serves as well).			
2. Silver pins, No. 20, ¼ oz.	0	2	0
3. Entomological pins, 1¼ in long, 1 oz	0	0	8
4. Cardboard (fine Bristol board), 4 sheets	0	0	3
5. Specimen tubes, flat, corked, each about	0	0	1
6. Pill boxes.			
7. A dissecting board, 12 x 3 in, half covered with black, half with white paper (made by self).			

Supplied by Messrs. C Baker & Co, London

INDEX

M

10 per cent of the sympathetic supply remains, the result is compromised 50 per cent. Another principle is that the operation must be performed in such a way that regeneration is avoided or minimized. In animals we know that the sympathetics will regenerate over long distances. Another principle that I believe is important is that the sympathetic system should, if possible, be interrupted in the preganglionic rami, leaving intact the postganglionic neurons. Cannon has demonstrated that if the postganglionic neurons are intact, there is much less sensitivity of the blood vessels to circulating epinephrine than occurs if these neurons are interrupted. Often, it is easier to divide the peripheral neuron than the proximal one in performing a sympathectomy, that is, it is easier to interrupt the postganglionic than the preganglionic neuron. The surgeon must appreciate this principle and direct the procedure toward the proper neuron.

A brief review of the anatomy of the sympathetic system and the splanchnic area will explain the principles employed in the operations for hypertension. The entire sympathetic system has its origin from the first thoracic to the second or third lumbar segments of the cord. The first, or preganglionic, neurons, on leaving the cord, traverse the anterior spinal roots and join the thoracolumbar ganglionated chain, which lies in the paravertebral gutter anterior to the transverse processes of the vertebrae. It is believed that the chief sympathetic supply to the large vascular bed in the splanchnic area arises from the sixth thoracic to the second lumbar segments. The preganglionic neurons connect directly or have an intermediary neuron to the celiac and adjacent ganglia in the region of the renal pedicles. From this latter group of ganglia, the last, or postganglionic, neurons arise and supply the splanchnic vasculature.

The thoracolumbar sympathectomy now in use removes all the ganglionated chain from the eighth thoracic to the third lumbar ganglia as well as the greater, lesser, and least splanch-

nic nerves. The white rami of supply are divided close to the points at which they emerge from the intervertebral foramina and brain clips are placed across the divided ends to discourage regeneration. All connections with the celiac and adjacent ganglia are cut, thus leaving these ganglia and their postganglionic neurons intact, although completely detached from any central control.

For adequate operative exposure, the lower ribs must be resected and the diaphragm divided. Only one side can be *done at a time, and the operation therefore requires two* stages, allowing about ten days to elapse between stages. Intratracheal ether is the anesthetic of choice and pressure anesthesia is resorted to only occasionally when the pleura is accidentally opened. A pneumothorax at the time of operation presents no difficulties and is corrected by aspirating the air as the wound is closed.

The results of this operation have not been completely tabulated by any means, but we have now 100 cases that have been followed for six months or more, and for that period of time we have been able to draw certain conclusions.

In the 100 cases operated upon six months to three years ago, two patients died postoperatively. This makes an operative mortality of 2 per cent. The total of operations performed to date now exceeds 150, and there have been no further deaths. The two patients who died were among the first 50 to be operated on and were what I would consider now to be bad risks. *One of them had cardiac decompensation and had been* digitalized; the other was so obese that a postoperative atelectasis which caused her death could not be satisfactorily dealt with. Twenty-four per cent can be classed as having a good result; that is, their blood pressures in any position are not more than 150/100. We have operated on no patients who have had "borderline hypertension." Thirty per cent show *improvement in that the blood pressure has been significantly* lowered from its original level and the highest level in any

position now is 165/110. In this latter group the postoperative pressure represents a lowering of at least twenty points in the systolic and at least ten points in the diastolic pressures over those present before operation. The two groups together comprise 54 per cent of the total and may justly be considered to have a satisfactory result from operation. This estimation of results is based entirely on lowering of the blood pressure. It *has nothing to do with the question of symptoms.*

Another 23 per cent have shown some sustained lowering of pressure but not enough, I feel, to warrant enthusiasm. Included in this group, for example, would be a patient who had a preoperative pressure of 280/160 and postoperatively 200/130. Perhaps it is unfair not to consider such a case as having been improved, but if this were as much as could be accomplished in all cases, there would be little justification, in my mind, for continuing the surgical treatment of hypertension. Twenty-one per cent had poor results; that is, there was no significant improvement.

The results in these 100 cases approximate those reported by Smithwick in 1943. In the 75 cases he followed one to five years after operation, he estimated that 61 per cent were improved, 16 per cent were slightly improved, and 23 per cent had poor results. The operative mortality was less than 3 per cent. It may be that the 2 or 3 per cent operative mortality can be lowered if there is more careful selection of cases, but even so it is apparent that the operation is a relatively safe procedure when you consider that most of these patients are usually considered poor risks for any operation.

There are various ways to evaluate results and much statistical study may be necessary before we can learn what we should know about the results of sympathectomy in hypertension. At present, we are trying to reason backward from our good results and our poor results in determining what patients are suitable for the operation. Now, we must assume that all the patients we subject to operation have about an

RESULTS OF OPERATION IN 100 CASES OF HYPERTENSION

No. of Cases	Grade of Eye Changes	Improved	Moderately Improved	No Improvement	Postoperative Death
		Type I Hypertension			
11	I	9	0	2	0
11	II	7	0	4	0
4	III	2	2	0	0
8	IV	6	2	0	0
34		24	4	6	0
		Type II Hypertension			
13	I	9	2	2	0
19	II	12	4	2	1
14	III	4	6	4	0
5	IV	2	3	0	0
51		27	15	8	1
		Type III Hypertension			
4	I	1	1	2	0
4	II	1	1	2	0
4	III	1	1	2	0
3	IV	0	1	1	1
15		3	4	7	1
		TOTALS			
100	...	54	23	21	2

equal chance for improvement. Some of those whom we were least enthusiastic about before operation have had the best results, and the reverse is also true.

White and Smithwick suggested an analysis of operative results based on the preoperative grade of eyeground changes and the type of hypertension. The universal division of eyeground changes is into four grades. In grade I there is narrowing of the arterial caliber; in grade II there is also arteriovenous nicking; in grade III there are retinal hemorrhages; in grade IV there is papilledema. The types of hypertension, of which there are three, are based on the following: Type I is that in which the pulse pressure is less than one-half the

diastolic pressure; type II is that in which the pulse pressure is equal to or not more than 20 mm. greater than one-half the diastolic pressure; and type III is that in which the pulse pressure is more than 20 mm. greater than one-half the diastolic pressure. Type I is presumably the most favorable and type III the least favorable for operation. White and Smithwick subjected 100 postoperative cases to an analysis in an attempt to discover what value the grade of eyeground changes and the type of hypertension might have in anticipating the operative results. I have similarly subjected my cases to this type of analysis and found results similar to those of White and Smithwick.

It may be seen that the percentage of good results from operation is highest in those with minimal eyeground changes and type I hypertension, but there are some failures. Also, in those with more advanced eyeground changes and type III hypertension, while the percentage of good results is low, there are some successes.

This study is presented in some detail to demonstrate how inadequate, thus far, our criteria are in determining accurately beforehand what results may be expected from operation. While generalizations can be made, based on such signs as eyeground changes, type of hypertension, and other aspects of the disease, evaluations of this kind may not pertain to the individual patient. Therefore, for the present, we choose to consider all hypertensives suitable for operation unless they demonstrate certain specific complications of their disease or some unrelated physical state not compatible with the operative risk.

Let us consider some factors that appear to be useful in determining the desirability of operation. I believe, first of all, that patients who have serious cardiac disease should be excluded. That kind of statement, I know, would be immediately challenged by cardiologists, but I have my own ideas about what constitutes serious heart disease. Patients

with heart block or cardiac decompensation or those who have had frank clinical coronary occlusion are very poor operative risks and stand to gain little from sympathectomy. Second, patients with advanced renal damage, i.e., those who have nitrogen retention which is not improved with the ordinary measures, or who show poor urine concentration, or who have a phenolsulfonphthalein output that is alarmingly low, are excluded from operation. Third, patients who show signs of acute encephalopathy, increased intracranial pressure, mental confusion, and disorientation should not be operated upon. Patients who have had strokes but have none of the other signs of cerebral disease, on the other hand, often are among those obtaining the best results from operation. In addition, it is found that patients over the age of 50 have uniformly poor or only moderately good results. The best results have been obtained in patients under that age.

Men, on the whole, respond less well than women to the operation, although some men have had remarkably good results. Two men in our series of cases have been threatened with induction into the army since their postoperative blood pressures met the requirements of army standards, and letters certifying their previous hypertensive states have been requested by the inductees desiring to avoid military service. Some of the poor or mediocre results in men in the past have doubtless been due to the policy of resecting the lumbar sympathetic chain on one side only, in order not to sterilize the patient. Resection of the lumbar chain to or below the second ganglion causes loss of ejaculatory power, though all other sexual functions are undisturbed. I am convinced now that failure to resect the lumbar sympathetics compromises the results, and I have taken the attitude, therefore, that unless a man is willing to have the complete operation, none should be done.

A few patients known to have glomerulonephritis have been subjected to thoracolumbar sympathectomy and, al-

though the blood pressure in each has been significantly lowered, they have not been bettered in other respects sufficiently to warrant the procedure. On the other hand, a few have been unsuspectingly operated upon whose kidney biopsies have shown them to have glomerulonephritis, and some of them have had a good lowering of the blood pressure.

Poor operative results have occurred consistently in patients who have shown a high diastolic pressure, over 140, particularly in those with significant impairment of kidney function. Poor response to both cold-pressor and sedation tests probably constitutes a contraindication to the operation, but I have not yet seen fit to deny operation to an eager patient on this basis alone.

We must finally show that in addition to lowering the pressure there are other benefits to the hypertensive state resulting from sympathectomy. The improvement in eyegrounds is a very constant finding following the operation. If there has been any lowering in pressure, hemorrhages will almost always disappear, and papilledema can be counted on to disappear if the pressure falls. One may reason from these changes that if the retinal vessels can change to this degree, perhaps the cerebral vasculature will also improve, since we usually look upon the ocular vessels as an index of the state of the cerebral vasculature. If possible, we should show that some improvement occurs in the cardiac status and in the renal status. Very little evidence has been seen that renal function is improved even when blood pressure is returned to normal, although Smithwick has reported such an occurrence. A few patients have appeared to show significant improvement in their cardiac status; *that is, improvement in* the electrocardiogram and in the size of the heart. One patient, whose electrocardiogram showed significant coronary changes before operation, a year later, with a normal blood pressure, showed a comparatively normal cardiogram.

In brief conclusion, therefore, I believe it can be said that

the thoracolumbar sympathectomy now in use gives promise, thus far, of producing a significant lowering of the systemic blood pressure in at least 50 per cent of the patients. We are beginning to obtain some evidence also that the lowering of pressure can improve the cardiac status, cerebral vasculature, and, very occasionally, renal function. The final proof of the value of the operation will come after the cases have been studied for a good deal longer than has yet been possible.

Dr. Cattell: Will you discuss the changes in cardiac status, Dr. Stewart?

Dr. Harold J. Stewart: It was only a short time ago that I looked over the series of electrocardiograms of one of these patients. Before operation there were progressive changes in the electrocardiograms which made us think that she had an anterior apex lesion. There was deep coving of the T-waves in Leads I, II, and IV. Serial changes occurred while the patient was under observation before the operation. A year later the T-waves were upright and of normal contour in Leads I, II, and IV.

Dr. Charles H. Wheeler: Might not these changes represent the recovery from coronary occlusion rather than beneficial effects of the sympathectomy?

Dr. Stewart: That is true. Patients show the same changes as the result of a coronary occlusion, so that one cannot be certain that the changes in this case are related to the restoration of the normal blood pressure. However, Dr. White recently reported a review of Dr. Smithwick's cases, and I think that in these there are enough observations showing that significant electrocardiographic changes follow the operation in many patients.

Dr. Harry Gold: I would like to ask a question about the cases of congestive heart failure which, you indicated, were not favorably influenced by the sympathectomy. By including them among the "poor results," do you mean that the blood pressure did not fall in these cases?

Dr. Ray: All the results have been evaluated in terms of blood pressure fall alone.

Dr. Gold: It isn't clear to me why, in patients with heart failure, the blood pressure should not fall after the operation in much the same way as in other cases unless it turns out to be that those who show heart failure also have such advanced vascular disease that the operation fails to lower the pressure.

Dr. Ray: There is, of course, the fact that few patients with congestive heart failure have been operated upon. My impression is that not only is the operative risk unduly great in such patients, but that significant improvement in their cardiac status or in longevity is hardly to be expected even though the blood pressure might be lower.

Dr. Gold: It may well be that the operative risk in patients with heart failure is much greater, but it isn't quite clear to me why such patients, provided they survive the operation, should not do better if their bood pressure falls following the operation. Excessively high pressure certainly increases the strain on the heart. But again, as you indicated, it may be that the number of such cases has been too few to make a final judgment.

Dr. Ray: All operative results cited here today referred only to the effect of the operation in lowering the blood pressure and disregarded the effect of the operation on symptoms. Since the operation is directed solely toward lowering pressure, the most pertinent evaluation of results can be based only on this change. Few, if any, patients are made more than temporarily worse symptomatically by the operation; many maintain that they feel better after operation even though pressure is not significantly improved, while some with good lowering of pressure find no improvement in those symptoms which they formerly associated with their hypertension. About 10 per cent of the patients that have been operated upon have had no preoperative (or postoperative) symptoms.

Dr. Eugene F. DuBois: I noticed that almost all of the con-

ditions mentioned as contraindications to sympathectomy represent types of patients upon whom one would not like to perform any major operation.

Dr. Ray: Hypertensive patients are notably poor risks for any type of major surgery. For example, the principal cause of mortality in cholecystectomy is hypertensive disease. It has been gratifying to find the operative mortality as low as it has been in sympathectomy.

Student: Is there a difference in the gastric acidity before and after the operation?

Dr. Ray: We have studied gastric acidity before and after sympathectomy and have found no effects. I have in mind, however, that section of the parasympathetic supply to the stomach might be more effective in diminishing gastric acidity. It would be a relatively simple matter to cut the vagi at the diaphragm. Both vagi are divided at the diaphragm often enough these days at the time of total gastrectomy and no known deleterious effects occur in the function of the remainder of the intestinal tract.

Dr. Janet Travell: It is interesting that Dr. Lester Dragstedt and his associates, of the University of Chicago, have reported that supradiaphragmatic double vagotomy in patients markedly reduces both the night volume of gastric juice and gastric acidity.

Dr. Harold E. B. Pardee: Have you any figures on the subsequent changes in the blood pressure after the early improvement?

Dr. Ray: I am not entirely prepared to answer that question. There is a common belief, which is frequently stated, that there is a tendency for the blood pressure to "creep" back up to its original level after an initial lowering, but some have felt called upon to qualify that statement. What I have found so far is that the blood pressure may gradually return to its preoperative level in the first six months, but if the pressure is at a lower level six months after operation, it tends to remain

lowered for as long as it has been observed. In other words, if the results are not evaluated until more than six months after operation the tendency for the blood pressure to "creep up" is not so impressive.

Dr. Pardee: How long after six months have you followed the blood pressure changes?

Dr. Ray: Up to three and one-half years.

Dr. Pardee: And in how many of those has the blood pressure crept back after six months?

Dr. Ray: In comparatively few. However, in the early cases, that is, those subjected to the other types of operation, and now followed ten, eleven, or twelve years, it has been noted that those few who had significant lowering of pressure after six months all continued to maintain the same pressure thereafter.

Dr. Cattell: The natural history of the disease seems so important in the evaluation of these results. I wonder if Dr. Gold would comment on that.

Dr. Gold: If we are going to draw any deductions from the behavior of 100 hypertensive patients who have been operated upon and observed carefully during a subsequent period of six months to several years, we have to relate them to a similar number of cases without operation who have been similarly carefully observed. That seems obvious enough, but I am not sure that such control observations are made as well as they should be. In the work of the Research Committee of the New York Heart Association there have been assembled records of many hundreds of hypertensive patients in attendance at the cardiac clinics over a period of many years. Some of these have been charted, and they give some idea of how variable blood pressure may be in any individual. Most people know that there are sharp peaks and troughs in the blood-pressure record; the first time the patient is examined the blood pressure may be 170/100, and the second time, a few days later, or a few minutes or hours, 140/85. Once he

seemed like a hypertensive patient and the second time like a normal one. However, it is the broader sweeps in the life history of the hypertensive state which I believe are so commonly overlooked, namely, the periods of many months when the blood pressure is very high, followed by periods of many months when the blood pressure is much closer to the normal range. I have brought here several samples of such cases which I would like to pass around. They cover frequent blood-pressure readings over periods of many months to years. On any of these charts you will find points at which you may place an imaginary sympathectomy with very gratifying results. Here is a record describing a period of observation of five years with an average blood pressure ranging around 220 systolic and 130 diastolic. If you place this imaginary operation at the appropriate point, it will be followed by a blood pressure ranging around 160/105 on the basis of four readings taken in the subsequent period of approximately a year and a half. Of course, as you see, the blood pressure went up again. These are random examples from a large batch of charts. They may not even represent the best ones. Here is an excellent record with the blood pressure gradually mounting, as shown by many readings taken over a period of a year and a half, with a systolic pressure between 190 and 200; now place the imaginary sympathectomy at this point and the blood pressure goes down to a level around 150, at which it remains over a period of more than a year. Before one can be impressed by the long-range hypotensive effects of the sympathectomy, an attempt will have to be made to match the results against such records as I have here.

Dr. Wheeler: There are many things one would want to know about these patients. How do we know that they have not had a coronary occlusion or are patients in congestive heart failure?

Dr. Gold: These patients have been very carefully studied clinically and with electrocardiograms. Those that I am

showing here are free of any factors that one can put one's hands on to explain the protracted period of lowered blood pressure.

Dr. Wheeler: Granted that this happens, as you pointed out, Dr. Gold, still it is unusual to see a patient with hypertension of long standing lose the hypertension, and it would be still more unusual, as in Dr. Ray's cases, to see a long series of hypertensive patients whose pressure happened to fall coincident with the operation.

Dr. Gold: I agree that it would be very unusual to see a long series of hypertensive patients lose their hypertension coincident with an operation, but then I don't doubt that the blood pressure goes down as the result of this operation, and that in some it may remain down for a very long time. In point of fact, the operation may cause such pronounced fall of the blood pressure and impairment of the vasomotor control, that for some time the patient may be unable to be upright without fainting. The question is not about the immediate or very early effects of the operation on the blood pressure, but about the long, persistent lowering of the blood pressure. Here I am inclined to describe the operative results somewhat differently.

My impression is, on the basis of the natural behavior of hypertensive patients, that important and persistent decline of the blood pressure as the result of sympathectomy does not apply to nearly half of the cases, as the present surgical reports indicate, but rather to a very small proportion. The operation is apt to be associated with a protracted period of inactivity and some revision in the patient's attitudes and habits favorable to the sustained lowering of blood pressure. The patient who has risked his life in a serious operation to lower his blood pressure is a chastened person and is likely, for some time, to avoid involvements in sustained physical efforts, tension-provoking situations, or torrents of emotion. Many of these long-standing moderate reductions of the blood pressure

following the operation may, therefore, be due to these factors rather than to the direct effect of sympathectomy. While I am inclined to agree that there are cases in which the removal of the sympathetics is the factor responsible for successful and protracted lowering of the level of the blood pressure, I am of the impression that the specific effect of sympathectomy has little to do with the sustained moderate reduction of the hypertension which occurs in the majority of the so-called successful cases. It is my opinion that when remissions which occur spontaneously and those due to such accessory factors as I have mentioned, are subtracted, the number of successful "cures" by sympathectomy will turn out to be very small.

Dr. Ray: I am well aware that in some patients blood pressure does fall unaccountably with or without operation. In our own series we are in a position to compare the initial results of the present operation with those of three other types of operations for hypertension. If the patients (about 65 in number) that were subjected to the earlier types of operation for hypertension be used as a control group, a comparison of their initial results with the initial results in the patients subjected to the thoracolumbar sympathectomy leaves no doubt regarding the specific depressor effect of the latter operation.

Another point to emphasize is that, in my experience, it is rare to see any significant drop in blood pressure after the first stage of a two-stage sympathectomy. The lowering, if any, comes after the second stage is completed.

Dr. Gold: That is the point. No one can help being impressed with the initial effects. It's the long-lasting effects that do not seem to me very impressive. Isn't the remission resulting from the operation merely an interlude of no great consequence in the history of the hypertension in the great majority?

Dr. Cattell: How do you explain the failure of the blood pressure to fall after the first stage? Physiologically it seems rather surprising.

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Dr. Wheeler: Granted that this happens, as you pointed out, Dr. Gold, still it is unusual to see a patient with hypertension of long standing lose the hypertension, and it would be still more unusual, as in Dr. Ray's cases, to see a long series of hypertensive patients whose pressure happened to fall coincident with the operation.

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whether it prolongs the life span of the patient. Does it add to his comfort? It seems to me very difficult to establish any control to decide how much benefit has resulted. It would be necessary to pick comparable patients or else go back years to similar age groups with similar blood-pressure levels and symptoms and use those as controls to evaluate the benefit of the operation. With regard to the blood pressure itself, I am inclined to think a person can get along satisfactorily even with a high blood pressure. I have followed some patients now for as much as ten years who had a history of high blood pressure going back fifteen years. We ought to have some criteria other than blood-pressure records to evaluate this more accurately.

Dr. Pardee: Don't you think we have this in the electrocardiogram, the eyegrounds, and the change of heart size? Those are three definite things that can be observed.

Dr. Deitrick: I have seen a patient who had a very large retinal hemorrhage and a blood pressure of 200/110 five years ago, and today her pressure is 165–180/100. Her retinal hemorrhages and her headaches have disappeared. Why, I don't know.

Dr. Ray: Those are exceptional cases. We are talking about 50 per cent of the whole, and you are talking about the exceptional case.

Dr. Stewart: One would not find 25 out of 100 cases of hypertension that would show any such results.

Dr. Ray: If it can be shown that operation stops or slows the progress of objective signs of the disease, that would provide one method of evaluation.

Dr. Deitrick: It would be desirable to have control studies on patients with the elevated blood pressure as the only abnormality.

Dr. Ray: We cannot save patients in the last stages of the disease. Our effort should be to prevent or postpone that.

Dr. Ray: I don't believe so, Dr. Cattell, because I am impressed with the fact that it does not take much sympathetic supply to make up for partial loss.

Dr. Cattell: Is that related to the fact that sympathetic innervation overlaps and is still profusely distributed after cutting part of the supply?

Dr. Ray: I think so. In our published results of the investigation of sympathetic supply to the upper limb, it was found that the segmental supply to the limb arises from the second to the tenth thoracic segments. In a few patients with Raynaud's disease all anterior nerve roots carrying sympathetic fibers to one upper limb were divided while all but one were divided on the opposite side. Postoperatively, the hand on the side on which a single root had been left intact was found to be elevated in temperature only one-half as much as the opposite (completely sympathectomized) hand. In one case, the subsequent division and in another the temporary blocking with procaine of the single remaining root resulted in additional rise in temperature of the homolateral hand to a temperature equal to that in the opposite completely sympathectomized hand. This experiment, we concluded, demonstrated the necessity for interrupting all sympathetic fibers to the area one wishes to sympathectomize.

Dr. Cattell: How extensive do you think the distribution of that fiber was?

Dr. Ray: It was extensive enough to maintain the temperature of the hand 50 per cent colder than the opposite one, and when that remaining nerve was blocked out with novacaine or cut, the temperature equaled that in the sympathectomized hand.

Dr. John E. Deitrick: I would like to comment on one point. Your study was presented from the standpoint of the effect of the operation on the blood pressure, but you stated that there is as yet no proof of the benefit from the drop in blood pressure. What we are really interested in knowing is

necessary, a placebo, and one frequently finds that the same patient carries on almost free of disturbing symptoms.

Dr. Ray: I make no claims for the effect of the operation on symptoms, since the indications for, and the results of the operation are not based on symptoms. As a matter of fact, the patients who get the greatest lowering of blood pressure are sometimes the most uncomfortable for the first period of months afterwards until they can accommodate to the new pressure. Often the patient who comes back in a month or two after the operation and says he feels fine, is the one that has had no lowering of blood pressure, but were I to rationalize the operation on such flimsy claims, I would feel less secure in defending it.

Dr. Mack Lipkin: You described the contraindications to the operation. What are your indications at the present time?

Dr. Ray: Those who don't have the contraindications.

Dr. Lipkin: Is any individual who has had hypertension long enough to establish it by numerous observations and who does not fall into the group with contraindications, to be considered suitable for the operation?

Dr. Ray: Yes.

Dr. Wheeler: Am I correct in understanding that, if you saw a patient over a period of six months who repeatedly had a blood pressure of 170 systolic and 105 diastolic, you would operate on that patient forthwith?

Dr. Ray: I would give him the opportunity of it.

Dr. Wheeler: I would like to ask a question of Dr. Stewart as the person who usually sees the hypertensive patient before the surgeon does. To which patients do you recommend the procedure at this time?

Dr. Stewart: I would go by that list which Dr. Ray showed.

Dr. Wheeler: Any patient who has a persistent hypertension?

Dr. Stewart: Without contraindications, yes.

Dr. Deitrick: That is why I would like some controls to see to what extent that can be done.

Dr. Ray: There is the Keith-Wagener evaluation of life expectancy in hypertensive disease against which results of sympathectomy may be compared after a few more years have elapsed.

Dr. Stewart: I think the way these patients feel is also important. They feel like normal individuals when their blood pressure is down to normal.

Dr. Gold: I want to say a word about the electrocardiogram. We ought to consider the electrocardiographic changes which occur after the operation with great circumspection. The electrocardiogram of a patient with hypertensive disease is extremely labile. We have observed large series of electrocardiograms taken during periods of several years in the hypertensive patients of our clinics. It is not at all uncommon to find the tracing normal at one time, while at another time, T_1 or T_2 is inverted, or the RT segment is depressed, these changes occurring without any particular relationship to the state of the patient. They are similar to the electrocardiogram of patients with coronary artery disease.

In one patient a coronary thrombosis will result in marked changes in the tracing which remain permanently, but the patient is quite free of symptoms. In another, the changes may disappear and the electrocardiogram returns to normal, while the patient is almost incapacitated with anginal pain.

As for symptomatic improvement in patients with hypertension, the interpretation of this in sympathectomized patients needs even greater caution. We are all familiar with the common experience of the anxious hypertensive patient who can hardly wait for the reading to be finished before he asks what his blood pressure is. If you tell him it is 220, he is very much disturbed and continues on his way with no end of symptoms. Tell him it is 150, give him some reassurance, if

necessary, a placebo, and one frequently finds that the same patient carries on almost free of disturbing symptoms.

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Dr. Stewart: Without contraindications, yes.

Dr. Wheeler: Could I ask you the same question, Dr. Pardee?

Dr. Pardee: The operation is a new procedure, about which there is a great deal yet to be learned. I would hesitate to present it or recommend it to a person who had hypertension which was asymptomatic and which was not associated with evidence of progressive arteriolar changes. The person who was having symptoms or showed arterial changes which I thought might result in a cerebral hemorrhage or a renal complication or a cardiac complication, I would think suitable for operation.

Dr. Gold: You would then send them for operation when they are in the late rather than in the early stage of the disease?

Dr. Pardee: Yes. I would not use it as a preventive as yet, because I am not quite sure at the present time how effective it is. I would not like to subject anyone to a procedure in which there is a 2 per cent operative mortality.

Dr. Ray: Of course, it is an eminently fatal disease we are talking about.

Dr. Pardee: I know, but you can watch it for three or four years without doing the patient much harm.

Dr. Gold: And many of these patients live to ripe old age. The reason which is often given for operating on an early case of hypertension is that one can hardly expect the blood pressure to fall in an advanced case in which the vessels are so badly diseased. That argument doesn't appeal to me. I recently saw a 37-year-old man with very advanced hypertension, on whom a Smithwick operation had been performed about two years previously. At that time he had a blood pressure of about 260/130. He now has a blood pressure of 240/130. He was subject to headaches, shortness of breath, and anginal pain on slight provocation before the operation. The headaches are somewhat diminished, but then, he worked very hard up to the time of the operation and has

not worked since. The anginal symptoms have not improved. About six months after the operation he developed a cerebral hemorrhage with left hemiplegia, from which he has recovered. The operation seems to have been a complete failure as far as one can judge. The point I wish to make, however, is that the failure was not due to incapacity on the part of the blood vessels to dilate, for shortly after the operation the blood pressure was so low that it could barely be registered, and when the patient began to be up and about he had to wear an abdominal binder as well as binders on his legs in order to help him maintain an adequate blood pressure.

The fact seems to be, in this case, that the sympathectomy temporarily eliminated his capacity for vasomotor adaptations, but appeared to have done nothing to alter the basic course of the disease.

Dr. Cattell: Dr. Goldring, associate professor of medicine at New York University, has been interested in this problem, but he was unable to be present so he sent a statement, which I would like to ask Dr. Wheeler to read at this time.

Dr. William Goldring: There are two prime objectives in the treatment of hypertension. One is specific and aimed at elimination of the cause of the disease, the other is nonspecific and aimed at the alleviation of symptoms.

In the earlier days of its application it was widely felt that sympathectomy might prove to be a specific measure on the grounds that human essential hypertension is caused by primary renal ischemia, and that such ischemia might be eliminated by relief of splanchnic vasoconstriction. However, there is a growing body of acceptable evidence against the primary renal ischemic origin of human essential hypertension, and furthermore, studies before and after sympathectomy have clearly shown that the renal blood flow does not increase after the operation.

It seems quite clear now that sympathectomy must be considered a nonspecific method of treatment.

Whether or not one is impressed with the results to date depends upon his acceptance of partial or temporary reduction in blood pressure as an advantage to the patient. This implies that the blood pressure itself imposes a strain on the arterioles and on the left side of the heart. The question of the relationship between hypertension and arteriolar disease is still unanswered. There are some who believe that renal arteriolar disease *precedes and accounts for the elevation* of blood pressure; others advance evidence which appears to indicate that hypertension precedes and accounts for the secondary occurrence of arteriolar disease. And still a third group of investigators are of the opinion that elevated blood pressure and arteriolar disease are independent of each other, i.e., both vasoconstriction and its symptom, hypertension, on the one hand, and arteriolar disease on the other, may be unrelated, concomitant effects of the still unknown pressor mechanism. In this latter view, simple lowering of the blood pressure would not be expected to reduce the degree or extent of arteriolar disease.

The existence of these three hypotheses, indicating a fundamental difference of opinion, strongly suggests that any assumed advantage to the arterioles of lowered blood pressure must still be considered a matter of speculation.

The sparing effect of lowered blood pressure on the left side of the heart seems obvious and is a highly desirable goal, but even in this regard I would be more impressed with reduction of blood pressure to average normal levels than merely a lowered blood pressure still in the hypertensive range.

It should be remembered that blood pressure is a highly variable function, the method for its measurement is crude, and in particular, there is no direct relationship between the height of the blood pressure and the severity of the disease. It would be unfortunate if an estimate of the worth of surgery in hypertension should eventually rest on nothing

more secure or significant than measurement of the level of blood pressure.

Sympathectomy is a method without equal for the relief of intractable headache; but in theory and from clinical observation of both medically and surgically treated patients, I am impelled to the tentative opinion that sympathectomy has not been established as a sound treatment for this disease.

Undue enthusiasm for surgery now might conceivably result in some slackening of interest in the major problem in the therapy of hypertensive disease, namely, discovery of the causative mechanism and its management by specific measures.

Sympathectomy is a nonspecific symptomatic treatment. Pending final analysis of accumulating data, it should be considered as no more than a highly desirable clinical experiment.

Present uncertainty concerning the value of sympathectomy is no better exemplified than in the conflicting views expressed by different investigators; one current opinion is that the operation should be restricted to those with advanced vascular disease, since no other form of therapy is effective; the other is that the operation should be reserved only for the earlier stages of the disease, since no benefit can be expected after extensive vascular disease has appeared. While it appears to me that the latter view is by far the more reasonable, I am impressed with the lack of agreement among competent observers who have had actual clinical contact with both medically and surgically treated hypertensive patients. The inference must be that the results in surgically treated patients are open to various shades of interpretation.

Its final appraisal must rest upon one single crucial criterion, namely, the life span of surgically treated patients as compared with expected longevity without operation. This is admittedly a difficult and perhaps even impossible task. If the answer is ever to come it will be from continued application to the problem, and I for one will look forward to a more

definitive statement in the future. For the present, I am compelled to hold in abeyance any real enthusiasm for this method of treatment.

Dr. Cattell: Would you like to answer any of the points Dr. Goldring made, Dr. Ray?

Dr. Ray: There is no real conflict between Dr. Goldring's and my statement of the problem. I repeat that from the surgeon's standpoint the operation is designed primarily to lower the blood pressure in hypertensives, and if in ten, fifteen, or twenty years from now it can be shown that these patients live longer, then the value of the operation will have been proved. It is true that in some patients headaches are benefited by thoracolumbar sympathectomy even though blood pressure may not be lowered, yet headache alone can rarely, if ever, be considered a worthy indication for such an extensive operation. It is all well and good to look for the cause and the nonsurgical cure of hypertension, but after these many hundreds of years none has been forthcoming, so I hope the medical profession will bear with a few of us who are approaching an old problem from a new angle. If we don't start, how can we evaluate the results twenty years hence?

SUMMARY

Dr. Gold: The surgical treatment of hypertension was the subject of the conference this afternoon. There have been several surgical procedures, but the one which appears to have proved most successful in lowering the blood pressure is the double thoracolumbar sympathectomy. This involves the preganglionic severance of sympathetic connections to the central nervous system from about the eighth dorsal to the third lumbar segments. The operation abolishes vasomotor control to the splanchnic area and lower limbs. We have had the views of a surgeon with a fairly extensive experience in this form of operation. He maintains that a fairly significant lowering of the blood pressure may be expected in at least 50 per cent of

the patients, and called attention to evidence that the operation not only lowers blood pressure but affects the course of the disease in other respects, namely, improvement in the vessels of the eyegrounds, the heart, and possibly the kidneys. Widely divergent views were expressed. An internist with a large experience in the management of hypertensive disease indicated that there was no evidence that the sympathetics are important in the causation of the disease. He stated his belief that sympathectomy is a nonspecific symptomatic treatment, serving essentially to relieve the intractable headaches of the hypertensive patient. While there is general acceptance of the fact that the operation produces initial lowering of the blood pressure, it is indicated that the pressure tends to rise again in the majority, and that the persistent moderately lowered level of the pressure in many of these patients may well be due to the altered habits and attitudes of the patient who has subjected himself to a serious operation for the purpose of escaping the dangers of high blood pressure. It was pointed out that there are long periods in the life of the hypertensive patient when the blood pressure is very much lower than at other times, and that such variations in the natural history of hypertension complicate the interpretation of the surgical results.

There are divergent opinions as to what kind of patients might be most suitable for this operation. Some prefer to operate only on early cases and others prefer to reserve the operation for long-standing ones in which it is clear that the disease is not stationary and is progressing to more serious phases. It was pointed out that whether patients who have been operated upon will live longer than medically treated patients with hypertension, is the crucial question, and that concerning this we have, as yet, no information. There seems to be fairly strong belief that thoracolumbar sympathectomy is a highly desirable clinical experiment at the present time.

Evaluation of Local Antisepsis

Dr. McKeen Cattell: Today we will attempt to evaluate antiseptics, with special reference to the problem of securing local antiseptic action by the application of drugs or other treatment directly to the skin and other tissues. The extent to which such antisepsis may be attained, as well as the relative effectiveness of different procedures, are important but difficult questions.

The discussion will be opened by Dr. Modell.

Dr. Walter Modell: Many important advances which have been made in medicine have followed the introduction of antibacterial measures. Thus the pasteurization of milk, the treatment of drinking water, the sterilization of contaminated excreta, and the fumigation of ships' holds have saved countless millions of lives and have probably prevented disease in many more millions. In a lesser way statistically, but just as dramatically, the introduction of the aseptic technic in surgery, of the sterile glove, the sterile gown and drapes, and the sterile scalpel have changed the face of surgery. All these very effective measures kill bacteria before they reach the human host.

However, the destruction of bacteria by means of chemical agents after they reach the human host presents special problems, many of which have, up to only recently, completely defied solution. The systemic introduction of chemicals to destroy bacteria which have invaded the blood stream or the organs of the body met with practically no success until the development of the sulfonamides and penicillin. We had

grown accustomed to failure in the quest for systemic antibacterial agents.

The prospects had always looked better for the surfaces of the body, the skin and the mucous membranes. Materials used for these surfaces are referred to as local antiseptics or disinfectants. There is no sharp distinction between them, although the term "disinfectant" is more frequently employed to convey the notion of complete destruction of the organisms, while "antiseptic" is more often applied to mere reduction of growth and multiplication of bacteria. In relation to the compounds used for these purposes, the difference is often only a matter of concentration.

Faith in the efficacy of locally applied antiseptics or disinfectants is widespread. It seems like a simple step from the demonstration of powerful destructive action on bacteria in a test tube to a similar action on such bacteria invading the surface of the mucous membrane or the skin. However, the problems are rendered very difficult by the complexity of the structure of the skin and mucous membranes and the conditions attending the invasion of these tissues.

There is now ample proof that inferences drawn from test-tube experiments may not apply to skin and mucous membrane disinfection. While there is no doubt that drops of silver nitrate solution in the eye prevent gonorrheal ophthalmia in the newborn, and a few other similar examples might be cited, there is indeed a question whether the application of disinfectant agents to the skin or mucous membrane does not often do more harm than good. At any rate, the view that the use of drugs by local application for the destruction of bacteria may give us a false sense of security deserves abundant consideration.

The list of substances which have antiseptic properties outside the body is long. In *New and Nonofficial Remedies* about twice as many pages are devoted to the listing of the anti-infective substances as to any other group of drugs. Attempts

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to attack bacteria by different mechanisms have led to a multitude of different types of agents, such as acids, alkalis, oxidizing agents, halides, freely ionized metals, poorly ionized metals, benzols, alcohol, formaldehyde, and the dyestuffs. Our discussion will concern itself chiefly with those which are for the most part nonselective, and destroy protoplasm pretty generally. There are, of course, the special problems of penicillin and the sulfonamides, which are not general protoplasmic poisons, whose actions are more specific, and, while they are employed for local application, apparently with some success, they are not commonly classed among local antiseptics and disinfectants.

Let me first point out how some of these disinfectants and antiseptics act and the factors which undermine the test-tube efficacy of these agents when they are applied directly to the skin and mucous membranes.

Acids and alkalis change the pH of the medium in which the bacteria live; some dyes react with acid or basic radicals of the bacterial protoplasm; mercuric chloride precipitates bacterial protein, while the organic mercurials are thought to combine with sulfhydryl groups of protoplasm, and phenols, to combine with amino groups in the bacterial protein.

Many factors modify the antiseptic power of these agents. Time is an important element. No antiseptic acts instantaneously, but the speed of action varies. Thus, whereas the phenol coefficient of mercuric chloride is only 13.5 in five minutes, in ten minutes it rises to 175 and in thirty minutes to 550. The significance of the time element is increased when dilute solutions are used, yet these are the only kind of solutions which may be used in therapy. Many factors in local application conspire to limit the effective time of exposure to any particular solution, such as evaporation, absorption, chemical combinations, and dilution. The temperature at which the antiseptics act on bacteria is also important. In general the effectiveness increases with temperature in the same

type of curve as most chemical reactions. While it is possible outside the body to maintain the optimal temperature, in therapy, obviously, no such control is feasible. Specific concentrations of antiseptics have been found to be the most effective against bacteria, or to give the optimum relation between toxicity for the bacteria and for the host. Yet the same factors which make the control of the time of exposure to bacteria impractical, also seriously interfere with the maintenance of optimal concentrations. Thus, 70 per cent alcohol by weight is said to be a critical concentration. Yet a moment after application to the body this concentration is reduced.

Surface-tension characteristics of antiseptics are important in therapy. Lowering surface tension by adding reductants often increases the potency of a drug. This is said to be caused by favoring permeation or penetration, accelerating osmosis and diffusion, and making the surface reaction between bacteria and the antiseptic a more intimate one; therefore, lowering the surface tension produces the same effect as increasing concentration. On the other hand, it should be remembered that the interfacial tension between air and liquid, as surface tension is usually measured, may give no accurate information concerning the tension at the interface between the antiseptic and the bacterium.

The ability of antiseptics to penetrate varies considerably. *Antiseptics in the colloidal state have little penetrating* power, while the same substances in true solution have, in general, considerably more. Antiseptics which coagulate or precipitate protein penetrate more poorly than those which do not; therefore, the phenolic antiseptics usually penetrate more efficiently than mercuric chloride. Penetration in vivo is always an important problem, since the multicellular membranes of the skin and organs present a formidable barrier to all antiseptics. The presence of protein in the medium seriously embarrasses antiseptic action. Protein affords the bacteria mechanical and chemical protection. It prevents pene-

tration; it reacts with the antiseptic to render it inert, insoluble, or less toxic. Particulate matter may adsorb some of the antiseptic. While this effect is universal, some, such as the *chlorine compounds and potassium permanganate,* are far more seriously impaired by the presence of organic matter than others, such as the benzol derivatives. It hardly needs mention that organic matter cannot be avoided or in any way limited in therapy with antiseptics, and as a matter of fact, the situation which calls for the use of antiseptics is often that which is accompanied by abundant serum or pus.

In evaluating these agents several tests are applied which attempt to take these limiting factors into account. The phenol coefficient, which when first conceived, tested only the potency of an agent against the Hopkins strain of Bacillus typhosus under standard conditions of temperature and time, has been expanded and modified to test as well a variety of organisms, the effect of organic matter, and penetration of a gel. In addition, the effect of these agents on suspensions of leukocytes or embryonal cells is also tested to obtain a ratio of toxicity between animal and bacterial cells.

All these tests applied to a drug appear to give a well-rounded answer concerning the relative effectiveness of an antiseptic. Yet the expected efficacy is usually not realized when the antiseptics are given actual therapeutic trial. Some reasons for this failure have already been pointed out. Most antiseptics penetrate tissues poorly, yet most so-called surface infections are at least several cell-layers deep. The cell membrane is a greater barrier to penetration than the agar gel which is usually used to test penetrability in vitro. A study by Nungester and Kempf has a bearing on this point. They swabbed the tails of mice with cultures of streptococci. Then they dangled the tails in baths of one of several antiseptics to be tested. Finally the tails were amputated and implanted into the peritoneal cavities of the mice. Practically all died of peritonitis. Since some bacteria must have penetrated several lay-

ers of skin, this treatment was not effectual. This difficulty in penetration also accounts for the serious problem encountered in sterilizing catgut by any method other than heat.

The shape of the infected area is important. A simple experiment demonstrates this. It is easy to sterilize the walls of an ordinary test tube with any ordinary potent antiseptic; yet if the walls of the test tube are drawn out into many thin filaments, making a multitude of crevices, it becomes exceedingly difficult to sterilize the tube with the same chemicals. This type of wound-model indicates why the irregularities and crevices of infected wounds seriously retard the action of antiseptics.

Antiseptics may also destroy some of the body's defenses against infection. Thus Fleming has shown that when lysozyme, one of the natural antibacterial defenses, is mixed with solutions of chlorine antiseptics, the power of both is reduced. The result of such a combination, therefore, favors bacterial development. Fleming maintains that often the concentration of an antiseptic is quickly lowered after application to a level below that which is antibacterial but which remains for some time antagonistic to the natural defenses of the body.

The cells of the skin and organs and the leukocytes may also be killed by effective concentrations of antiseptics. Very few antiseptics indeed have a truly favorable therapeutic index, in vitro, killing or inhibiting bacteria at significantly lower concentrations than they do leukocytes. Thus most of them also kill some cells, irritate others, stimulate the flow of serum, and produce the best possible medium for the growth and multiplication of the bacteria which survive the initial antiseptic blow. There is evidence collected during the last war which showed that after contaminated wounds were débrided, irrigation with sterile saline produced cleaner wounds, and wounds which healed more quickly than those treated with any of a number of antiseptics. Indeed, impression cultures showed that the bacterial flora was most luxuri-

ant in those wounds treated with antiseptics, while it was very sparse, by comparison, in those merely irrigated with sterile saline.

All of the factors mentioned conspire to make what looks like an effective antiseptic not only useless but sometimes also harmful.

I would like to end with a quotation from Alexander Fleming written some twenty-five years ago. He referred only to surgery, but the same advice applies to all medical practice in which antiseptics are used. He said that the evidence "would seem to show that the antiseptic plays no part in the primary treatment of wounds. If this is so, then there is a very great disadvantage in the use of an antiseptic from the surgeon's point of view. It is very difficult for the surgeon not to be deluded into the belief that he has in the antiseptic a second string to his bow, and consequently it will tend to make him less careful in his surgical treatment of the wound. If he knows that he has nothing to fall back on, then, even with the most conscientious individuals, the surgery would improve. Because of this alone it would be well if the treatment of the *wound* with antiseptics in the early stage were abandoned and the surgeon relied on his skill alone. All the great successes of primary wound treatment have been due to efficient surgery, and it seems a pity that the surgeon wishes to share his glory with a chemical antiseptic of more doubtful utility."

Dr. Cattell: Dr. Modell has made some challenging statements. I don't know whether he is going to be allowed to get away with all of them. We have present men working in the fields of dermatology, surgery, urology, and medicine, and we would like to hear from them in relation to this problem.

Dr. Charles H. Wheeler: I wonder if we might ask Dr. Modell to define the problems a little more sharply?

Dr. Modell: I question whether any useful purpose is served in rubbing the skin with a little alcohol before introducing

the needle through it for a hypodermic injection, whether there is any value in swabbing an infected throat with an antiseptic, whether there is any use in irrigating an infected bladder with such agents, or the eye, or the vagina, or a wound. I think that these are for the most part useless gestures, sometimes detrimental, and that reliance on these attractive practices leads one away from better drugs and better technics.

Dr. C. Gardner Child: Probably no single technical subject in surgery has been the object of more attention than has this one. In attempting to further good surgical technic innumerable efforts have been made to discover some agent which would sterilize the skin through which an incision was to be made. I think that in general the remarks already made hold true. It has certainly been discouraging to discover that each of the chemical agents, as it has been placed in use, has proved to be ineffective or to do more harm than good. In a recent report the author found his results as good, in fact better than previously, when the only agent he used for preparing the skin was a bland white soap and water.

At the present time the Department of Surgery uses the following method of skin preparation. First, fat and grease are removed with benzene, followed by alcohol and ether. The skin is then painted twice with 3.5 per cent iodine, which in turn is removed with alcohol. Some degree of superficial sterilization of the skin is probably accomplished by this method, but certainly it does not extend to the deeper layers, into the hair follicles or skin-gland systems.

Traumatic wounds constitute a much larger problem. Here mechanical cleansing with copious amounts of saline and thorough débridement are the only methods employed in converting a contaminated wound into a clean wound.

From the surgical point of view I would agree almost entirely with what has been said this afternoon, namely, that the mechanical cleansing of the skin and wounds is about all

that is effective. The use of various chemical antiseptics accomplishes little and often is harmful.

Dr. John M. McLean: Do you use any soap on the skin?

Dr. Child: Yes.

Dr. Modell: In connection with skin preparations, Price has shown that after scrubbing the hands with soap and water for about ten minutes about 100,000 bacteria can still be recovered from the wash water. After sterile gloves have been on, for two or two and a half hours during an operation, the bacterial population may again be as high as it was before the scrubbing.

Dr. Bernard Maisel: Price also reported that the use of alcohol and soap facilitated the lowering of the bacterial flora of the hand.

Dr. Modell: But no measures make the hands absolutely sterile. Price has shown that antiseptics may fix bacteria to the skin of the hands but neither kill nor remove them. They may still be viable.

Dr. Harry Gold: Do you imply that alcohol used with the soap in scrubbing exerts an antiseptic action?

Dr. Maisel: I think that was a statement of Price in his report.

Dr. Gold: Isn't there something strange about that, since other reports show that it may take as long as sixty minutes for alcohol to kill the staphylococcus?

Dr. Modell: Price found that a specific strength of alcohol, namely, 70 per cent *by weight,* made an effective antibacterial washing solution. This concentration takes much care in preparation and is easily disturbed. For example, it is quickly changed during the course of its use as a wash. The slightest deviation from 70 per cent by weight—not by volume—makes it an ineffectual wash.

Dr. Gold: I would like to ask why we continue at the New York Hospital the time-honored mixture of alcohol and io-

dine in preparing the skin for operation? Is that just tradition?

Dr. Child: It probably is mostly a matter of tradition.

Dr. Cattell: Is not the color helpful?

Dr. Child: We take the color off.

Dr. Cattell: You do finally, but it tells you where you have been.

Perhaps we will take up some of the other aspects, and from here go on to the dermatologist, Dr. Sulzberger.

Dr. Marion B. Sulzberger: In his remarks Dr. Modell has defined "antiseptic" in a somewhat restricted sense and has also deliberately excluded from his discussion a number of recognized bactericidal or bacteriostatic agents such as sulfonamides, penicillin, etc.

For the purposes of my own comments I shall define an "antiseptic" in the usual or classic way, i.e., as given in standard medical dictionaries. The definition of an antiseptic, then, usually reads something like this: "An agent which prevents the action of germs which produce fermentation, putrefaction, or disease." I believe it must be granted that under this definition many agents used on the skin's surface or on wounds or skin infections act as antiseptics.

I say this despite my essential agreement with the facts which Dr. Modell has so well set forth, all showing that no known form of external application is capable of effecting *complete or perfect sterilization of the skin's surface without* damage to the skin itself. We have found that neither living skin in its natural position nor excised skin tissue can be completely freed of the presence of living micro-organisms by any form of application which does not destroy or seriously damage the living cutaneous tissue. When Dr. Rudolph Hecht and I made "skin antigen" for sensitization experiments and skin tests, we used many different bactericidal and bacteriostatic solutions including iodine, mercurials, and sulfonamides in our attempts to sterilize the excised skin and still

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inhibition of generation and of multiplication, and the fixation or mechanical removal which can be accomplished either by chemotherapeutic agents, by solvents, precipitants and fixatives (alcohol, etc.), by washings, or by many of the other external measures generally employed, all are worthy of consideration as practical antiseptic measures—provided one condition is fulfilled. This condition is that the measures used to remove, fix, or damage the micro-organisms have the proper *therapeutic index*, i.e., that they accomplish their effects in regard to the micro-organisms without producing too much damage to the skin tissue. This is of the very greatest importance in cutaneous antisepsis in relation to both prophylaxis and treatment of infection, for even slight degrees of skin damage may materially interfere with the usually adequate and remarkably efficient natural bactericidal and protective powers of the skin and its secretions. The *vis medicatrix naturae* of the normal skin is the most important factor in the prevention of cutaneous infections. We must preserve these protective powers at all costs. Any measure which radically disturbs or destroys the normal "acid mantle" or the skin's electrophysical barriers or the bacteriostatic properties of sweat or which in any other way interferes with the truly remarkable natural protective mechanisms is likely to do more harm than good, regardless of its intrinsic or in-vitro bactericidal or antimycotic activity.

This is one reason why there is so little relationship between the antibacterial or antimycotic activity which an agent may evidence in the laboratory, and the effects achieved by that agent when used on the skin. Another very striking reason for the almost constant discrepancies between in-vitro effects and clinical therapeutic effects is that the actual mechanisms by which agents applied to the skin cure or prevent microbic disease often have no relationship whatsoever to bactericidal or fungicidal mechanisms as manifested in-vitro.

Among the most important mechanisms operative in curing

preserve its characteristic specificity. We were unsuccessful, and bacteria remained viable in the excised pieces.

This fact is even more apparent when trying to sterilize the living skin on the body. Older studies and the more modern washing experiments of Price, cited by Dr. Modell, the experiments of Pillsbury, Livingood, Nichols, and Shaffer with wash waters, with other agents, and with sulfonamides, all prove this fact. We possess no measure or method which will destroy all the micro-organisms (many of which live and propagate within the horny layer and within the skin crypts and openings of the follicles and glands of the skin) without damaging the living skin cells. This is the basis for Dr. Modell's important conclusion *that there has been a rather general tendency to lean more heavily on external antisepsis than the facts appear to warrant.*

Nevertheless it would, I believe, be erroneous to conclude that local antisepsis is of no value whatsoever. Take for instance the soap-and-water-washing experiments of Price and the sulfonamide studies of Pillsbury and coworkers. These procedures produced a very considerable reduction in the number of bacteria on and in the skin. Dr. Modell, do you happen to recall the degree of reductions in numbers in Price's washings?

Dr. Modell: After ten minutes of scrubbing, the numbers were reduced from about ten million bacteria to about one hundred thousand in the last wash water.

Dr. Sulzberger: Such distinct reductions in numbers of micro-organisms must be considered as a demonstration of the possible antiseptic effect of soap and water and of other agents applied—for, granted a certain degree of natural protective capacity on the part of the tissues, the actual number of potentially pathogenic bacteria is a significant factor in overcoming tissue resistance—and reduction in their numbers may well prevent the action of germs producing disease. The damaging effect upon micro-organisms, the bacteriostatic effect or

inflammation, the pH tends to increase, and many skin lesions have a pH as high as 7.0, 7.5, or 8.0. Medicaments with acidifying and buffer effects, such as Burow's solution, combinations of organic acids and their salts (lactic acid, propionic acid, undecylenic acid, succinic acid, etc.) probably help to restore the normal degree of acidity and thus help to counteract infection.

It will be apparent that these few measures I have been able to list all act by aiding, and sometimes by intensifying, the natural antimicrobic protective mechanisms of the skin. These effects can therefore never be observed or studied through in-vitro experiments. They are, nevertheless, antiseptic effects—not, perhaps, by Dr. Modell's definition, but certainly by the classic definition of "agents which prevent the action of germs which produce disease."

In addition to these measures there are conditions under which the intrinsic, in-vitro bactericidal or bacteriostatic or fungicidal or -static effects of medicaments prove useful. Thus, for example, in treating impetigo contagiosa, the cure may be accomplished by aiding the natural resistance by merely scrubbing off the crusts and applying softening and occlusive measures, such as the application of an inactive grease like petrolatum. However, it is undeniable that the *autoinoculation of new sites* tends to be inhibited if a mercurial or sulfonamide or other such antiseptic is incorporated in the ointment or other topical therapeutic agent which is spread over the skin's surface and stands ready to catch the micro-organisms which are being disseminated from the sites of the original lesions.

In this manner the iodine or mercurial painted on a belly may serve to good purpose, not by destroying all the microorganisms already present in those skin areas, but perhaps by destroying the chance ones reaching the skin afterwards from the air or hands, etc. Moreover, if a physician's hands have been covered with a sulfonamide-containing cream, micro-

or preventing infection is that of removal of large numbers of pathogens from the skin's surface. One way in which this is accomplished is by dissolving, emulsifying, and washing off grease and horny material, sweat and dirt (the skin's soil) together with the micro-organisms which are intimately incorporated in this soil. Another and most efficient way of preventing micro-organisms from producing disease is by helping the skin itself cast them out. This can be done through the application of agents promoting desquamation. It appears to me probable that many of the medicaments we use in treating surface infections (and particularly in treating or preventing fungus infections) effect their benefits by promoting more rapid desquamation and thereby aiding in the removal of the pathogenic agents. This mechanism may well be the *modus operandi* of such common "antiseptics" as salicylic acid, benzoic acid, resorcin, mercurials, sulfur, tincture of iodine, etc.

Another manner in which infecting agents are removed is through the measures employed to soften and evacuate abcesses, or to remove crusts or secretions, etc., in which micro-organisms may be present or may thrive. Thus, compresses, wet dressings, keratolytic or softening ointments and plasters, soap and water, etc., all become "antiseptics."

Still another form of cutaneous antisepsis acts through mobilization and concentration of the natural protective agents of the circulatory and tissue fluids, bringing the phagocytic cells, *the antibodies, and other anti-infectious agents to the* site. This action is inherent in hot poultices, in "counter-irritants," etc.

A more subtle form of antisepsis is effected by measures which preserve or restore the normal hydrogen-ion concentrations of the skin's surface. In most areas the normal skin's surface is distinctly acid, having a pH between 3.5 and 4.5. It is obvious that this acidity is protective, since it tends to damage and impede pathogenic micro-organisms. As soon as there is the slightest abrasion, interruption of continuity, or

using will kill bacteria. They place great faith in the value of this action. I have taken the position that such an action is of little value under the conditions of actual practice, and may be harmful. I think that this is substantially what Dr. Sulzberger has stated.

Dr. Cattell: I hope we can discuss these problems further, but we have more ground to cover. Dr. Marshall, I wonder whether you would take up the use of local antiseptics in the urinary tract.

Dr. Victor F. Marshall: Dr. Modell mentioned the urologic procedure of irrigating the bladder and questioned whether it kills germs in the bladder. Recently a heroic attempt was made to sterilize the bladder, primarily with the idea of killing a virus which might be the cause of papillomatosis. Fifty per cent phenol and glycerine were introduced into the bladder. Whether the patients so treated will get papillomas is very difficult to determine, because they have had so much trouble. However, the treatment did not sterilize the inside of the bladder. In fact, invariably these patients developed infection as a result of the damage to the bladder wall.

It has been shown numbers of times that it is difficult to infect a normal bladder, that is, one which empties itself well. If you put virulent cultures into the bladders of dogs, only a small percentage develop cystitis, and those cases clear up rather quickly. But if you injure the bladder, by poking or scratching the surface, or radiating with x-ray or radium, the same cultures infect readily.

In irrigating the bladder we don't think we are sterilizing the surface of the bladder wall. We know we are not. Several beneficial things are accomplished, however. Primarily, it constitutes a mechanical cleansing of the bladder. Infected bladders usually have a great deal of mucoid matter, pus, etc., which is washed out. Stagnant urine is removed. Drainage is promoted, and any infection which drains well is more likely to be taken care of by the body forces. The other thing which

organisms which leave those hands may carry with them their own pharmacotherapeutic nemesis.

What I have said is perforce fragmentary, but I hope that it will serve to bring out three principal points:

First, that the mechanisms through which antisepsis in the wider sense is accomplished on the skin, are not usually through the intrinsic antiseptic properties of the agents applied, but rather by means of aiding, intensifying, or restoring the skin's natural protective devices.

Second, that all measures used in the antisepsis of normal skin must possess an absolute minimum of harmful effects upon the skin itself, otherwise the interference with powerful, natural protective forces will often vitiate any intrinsic antiseptic properties the measures may possess.

Third, that the recognition of the above facts inevitably leads to the conclusion that studies on skin antisepsis (including the evaluation of old remedies and the development of new ones) require a drastic reorientation. In order to gain valid information regarding desirable and undesirable effects of cutaneous antiseptic procedures, it is imperative to study by all possible technics the effects accomplished and the changes brought about when the remedies are applied to the living human skin, rather than to study their effects on other organs, or in fluids or cultures or test tubes.

The therapeutic index of a cutaneous antiseptic measure cannot be established except on the skin itself, for both antiseptic activity and harmful effects often depend on mechanisms which are peculiar to the living skin *in situ* and on conditions which cannot be duplicated elsewhere.

Dr. Modell: I find myself for the most part in agreement with Dr. Sulzberger. He has pointed out that chemical agents may act in many ways to rid the skin of bacteria, and that these may include materials which have little or no power to kill bacteria. Most people who dab something on the skin to prevent infection have in mind that the substance they are

wise, with external ocular infection, particularly conjunctivitis, there seems to be little room for argument as to what is effective and what is not, and in the more serious cases of conjunctivitis there seems to be nothing chemical which can be introduced into the eye which is anywhere nearly as effective as repeated simple irrigations which remove the excess pus and débris which tend to collect in the eye and impede normal drainage. I must except, of course, as we did in the beginning, the sulfonamides and quite likely the mold extracts.

It is very true, though, that there is a long list of local antiseptics which may be dropped into the eye. I can't begin to remember them all. Under various trade names, they include many metallic salts, both inorganic and organic, particularly those of silver, mercury, copper, zinc, and some benzene ring preparations. By and large they are sufficiently diluted so they don't do very much harm, but I don't believe you can say much more for most of them. The irritant properties of many of them help to increase the flow of tears, and thus enhance cleansing.

There is one apparent exception in the case of angular conjunctivitis. This is caused by the Morax-Axenfeld bacillus, which secretes a proteolytic enzyme. The symptoms are promptly arrested by the use of a solution containing a zinc ion (usually the sulfate or the chloride). However, the action is not bactericidal, for the bacillus grows well in culture media containing zinc. The zinc ion has its symptomatic effect by inhibiting the enzymatic proteolysis.

So far as sterilization of the eye and the surrounding skin before operation is concerned, that is another problem. We use approximately the same technic as Dr. Child outlined for preparing the surrounding skin of the lids, face, and brow, and we use simple irrigation of the conjunctival sac in the hope that we will mechanically get rid of some of the organisms which may be there. We do use mild silver protein just before irrigation, not because we believe that silver protein

has to be remembered is that a large number of our patients have residual bladder urine. Organisms apparently multiply rapidly and very well in the warm residual urine. We believe that, if you leave some mild antiseptic in the bladder, the residual urine will have bacteriostatic properties that it would not otherwise have. In short, we don't do bladder irrigation with the idea of sterilizing the bladder by the action of the irrigating agent.

In urologic surgery we sometimes use only soap and water for skin preparation. This is most frequently done in operations on the external genitalia. One reason is because in these areas blistering occurs easily after the stronger chemicals which we use on the skin elsewhere. I have no impression that in those instances there is any greater incidence of infection than in those patients prepared in the standard manner. We routinely use soap and water preparation for transurethral resections, frequently ligating the vasa at that time, and I don't recall here either that the incidence of infection is higher than in those done after alcohol and iodine preparation.

The other question is that of the sterilization of the urethra. It apparently cannot be done without damaging the urethra. There have been a number of attempts made to do this. You can reduce the bacterial population by irrigating, but you cannot get rid of the organisms completely or permanently. It is much like the skin.

Dr. Cattell: There is one more organ which we would like to have considered before we ask for general discussion. I wonder whether Dr. McLean would say something about the possibility of sterilization in the eye.

Dr. McLean: There is not very much to be said which has not already been said. Dr. Modell mentioned the outstanding exception, the accepted Credé method for preventing ophthalmia neonatorum, and there is no question but that it has reduced the incidence of ophthalmia neonatorum. Other-

skin should be a gentle procedure, because anything which tends to cause a serous ooze defeats the purpose of cleansing. We saw that beautifully demonstrated in the laboratory not so long ago in experimental animals.

Dr. Modell: Dr. Child, how do you prepare the field in the region of the rectum for, let us say, a hemorrhoidectomy? What skin-sterilizing procedures do you use there?

Dr. Child: I use nothing more than soap and water. Others apply various dyes in a vehicle which will not cause a chemical dermatitis. Soap and water, I think, is the most desirable preparation.

Dr. Modell: This would indicate that soap and water will serve satisfactorily in the preparation of a most highly contaminated operative field in a surface full of crypts and folds. It should, therefore, be adequate for the preparation of the abdomen.

Dr. Sulzberger: I think that this may be one of the rare occasions on which I can come to the aid of a surgeon. When one removes "soil" by scrubbing with soap and water it requires a certain amount of mechanical energy or friction. The amount of friction necessary may perhaps be reduced by using a combination of grease solvents and soap and water washings. The dissolving of the grease and the softening of the film in which the bacteria and other elements of the soil are imbedded permits the easier emulsification and washing off by soap and water.

Dr. Cattell: Isn't soap more effective?

Dr. Sulzberger: I believe that either grease solvents or soap-and-water washings soften and remove soil even when used alone, but that with their combination you may perhaps get a sort of synergistic effect, which requires a minimum of friction and skin damage while effecting a maximum degree of surface cleansing and removal of micro-organisms.

Dr. Cattell: Those are agents which are not desirable from the standpoint of the skin, such as benzene and alcohol.

in one form or another is going to sterilize anything, but because it makes it easier both mechanically and visually to insure a thorough irrigation, which is made until the returns are clear, using the silver protein as an indicator.

I would like to ask Dr. Modell one question. If you were to undergo a major surgical procedure, how would you like your skin prepared and how would you like your surgeon to prepare his hands?

Dr. Modell: I would want the surgeon to take every precaution not to introduce more bacteria into my body than were already there. The instruments, the drapes, the dressings, and all the other paraphernalia should be made just as sterile as possible. I don't doubt that the washing of the surgeon's hand is desirable, simply on the basis that the gloves might tear. I certainly would want him to wear sterile gloves when he operates on me. I would demand a good cleansing of my skin with soap and water, which is, I think, about as good a preparation of the skin as can be obtained.

Dr. McLean: Would you like to have his hands soaked in alcohol or any other solution either before or after washing?

Dr. Modell: I wouldn't care.

Dr. McLean: Would you object to it?

Dr. Modell: No, I don't think it would make any difference as long as they were washed for ten minutes.

Dr. McLean: Would you object to anything besides soap and water on your skin?

Dr. Modell: I don't think painting with a colored substance is at all harmful.

Dr. McLean: How about the fatty solvents, benzene, ether, and what not?

Dr. Modell: I think they are unnecessary.

Dr. McLean: I am trying to find out what you would want.

Dr. Modell: I would insist on a cleansing with soap and water. I don't think anything else accomplishes more.

Dr. Child: Could I comment on one point? Scrubbing the

Dr. Cattell: Except for the extra trouble, it appears that the results would be probably better from soap and water.

Dr. Wheeler: I don't think they could be better, because the results from alcohol are perfect.

May I ask a question? Dr. Sulzberger, in a disease like impetigo, a bacterial infection of the skin, do you feel that the use of local applications is worthless?

Dr. Sulzberger: No, the use of local applications is surely valuable, but a good deal of what they do is mechanical. It has been demonstrated that the vigorous use of soap and water and petrolatum sometimes brings results just about as good and as prompt as white ammoniated mercury. Furthermore, white ammoniated mercury without measures which remove the crusts does little more than inhibit autoinoculations.

Dr. Gold: Do you think we could get general agreement on the need for at least one year's experience in New York Hospital with the use of soap and water for all kinds of skin preparations in all situations in which antiseptics have been used in the past for presentation at another conference next year?

Dr. Cattell: It would be fine if we could. Apparently surgical technics are already going along those lines.

SUMMARY

Dr. Modell: A therapeutic procedure practiced more widely than any other by both physicians and the laity is the application of a chemical for the purpose of destroying bacteria on the mucous membranes and the skin. The agents referred to as local antiseptics or disinfectants are used in the form of swabs, instillations, irrigations, sprays, etc. The spot is dabbed with alcohol before every injection. The skin is painted with iodine and washed with alcohol, or treated by some other antiseptic, before surgical operations. The bladder is irrigated with an antiseptic wash. The conjunctiva is flushed with a solution containing an antiseptic. There are numerous other practices of a similar nature. The discus-

Dr. Sulzberger: Of course, organic solvents may often be harmful because they remove too much of the natural oils and skin greases. This holds true particularly on skins or in skin areas which have an inadequate grease-producing capacity or when the solvents are used too long or at oft-repeated short intervals.

Dr. Maisel: I don't know why sulfonamides and penicillin were excluded from the discussion, for it seems to me that those are the agents which are now becoming most popular.

Dr. Modell: I believe the sulfonamides will stand by themselves and so will penicillin and other antibiotics. This discussion should serve to emphasize their importance by helping to eliminate a lot of chemicals now used. From the report of the pharmacist, Mr. Clarke, we learn that last year we used for the hospital, not including the college, about 4,000 gallons of alcohol. The alcohol shortage is still fairly acute. Its usefulness in preparing the skin before hypodermic injection is dubious at best, and certainly useless when we consider that no one allows it to remain for more than one instant before the needle is plunged into the skin. Does anyone think we have a vital procedure here? Does it do anything more than make the needle-prick hurt more than it would without alcohol on it?

Dr. Wheeler: I think anyone who cleans the skin with alcohol before injecting should not think he is sterilizing the skin but rather that it is just a convenient and good-smelling way of cleaning it. I would be willing to use anything else if it were equally convenient. Soap, I am sure, could be used with equal results.

Dr. Modell: I would like to point out that it is a fairly common procedure for the nurse to put an alcohol sponge on the sterilized needle while it waits for the doctor. Since bacteria can be cultured from most of the 70–80 per cent alcohol used, this little bit of nursing technic reduces the sterility of the whole injection procedure.